TWO DECADES OF PROGRESS
THE UNITED STATES-JAPAN COOPERATIVE MEDICAL SCIENCE PROGRAM

The Fourth Five Years
1980-1985

The United States-Japan Cooperative Medical Science Program

Fourth Five-Year Report
1980–1985

DEPARTMENT OF STATE PUBLICATION 9429
East Asian and Pacific Series 219

Bureau of Oceans and International Environmental
and Scientific Affairs

Released July 1985

I

OFFICE OF THE PRIME MINISTER
TOKYO

MESSAGE FOR THE UNITED STATES-JAPAN COOPERATIVE MEDICAL SCIENCE PROGRAM

In January 1965, the U.S.-Japan Cooperative Medical Science Committee was founded in accordance with the Joint Communique issued by President Johnson and Prime Minister Sato. It gives me great pleasure to note here that, favored by the close and extensive cooperation of a number of prominent medical scientists from both countries, the Committee has been making constant progress, and this year the Committee celebrates its 20th anniversary.

I believe that the progress made in the past five years, which was summarized in the form of the Fourth Five Year Report, will receive great appreciation as the result of the U.S.-Japan cooperation in the field of medical sciences for such a long period as 20 years.

The history and achievement of this Committee toward the attainment of the worthy cause of elimination of diseases which still prevail in Asia show the steps of cooperative spirit between the United States and Japan. It is quite meaningful as an evidence of our friendly relationship, which has contributed to the improvement of the welfare of the Asian people.

On behalf of the Government and people of Japan, I wish to take this opportunity to extend my hearty congratulations and to express my deep admiration to all the people concerned, including the Committee members, for their devoted efforts in promoting such eminent undertakings in spite of the various difficulties involved.

Finally, I would like to express my best wishes for the furtherance of the Committee's activities and cooperative programs and believe that these activities and programs will continue to make significant contributions toward peace and welfare for all mankind.

Yasuhiro Nakasone

Yasuhiro Nakasone
Prime Minister of Japan

THE WHITE HOUSE
WASHINGTON

MESSAGE FOR THE UNITED STATES-JAPAN COOPERATIVE MEDICAL SCIENCE PROGRAM

On behalf of the government and the people of the United States, I wish to extend my hearty congratulations to you on the occasion of the Twentieth Anniversary of the United States-Japan Cooperative Medical Science Program.

This program which was founded by President Johnson and Prime Minister Sato in 1965 exemplifies the achievements that can be attained when dedicated scientists from our two great nations join together in an effort to alleviate the human suffering caused by disease and malnutrition.

Over the years, this program has been guided by an enlightened joint committee that has had the foresight in directing research programs to take advantage of significant new discoveries and developments that have occurred in the biological and health sciences over the past two decades.

As this most successful bilateral program moves forward into its third decade, we look forward to continued cooperation between the scientists of our two countries as they explore the medical and biological sciences and appropriate biotechnologies to combat, contain or eradicate disease and to advance the state of human health throughout the world.

Ronald Reagan

Ronald Reagan

CONTENTS

Page

Preface v

Dedication vii

Membership of the Joint Committee viii

Secretariats xi

Drafting Committee xiii

Joint Communique xv

Introduction 1
  Organization and History 1
  Activities of the Joint Committee 1
  Guideline Changes, 1965–85 3
  Criteria for Panel Review 15
  Panel Reviews 15
    Leprosy 15
    Parasitic Diseases 16
    Viral Diseases 17
    Tuberculosis 18
    Hepatitis 19
    Environmental Mutagenesis and Carcinogenesis 20

Research Reports
  Panels

    Cholera 21
    Environmental Mutagenesis and Carcinogenesis 55
    Hepatitis 77
    Leprosy 101
    Malnutrition 134
    Parasitic Diseases 157
    Tuberculosis 203
    Viral Diseases 237

  Board

    Immunology 263

PREFACE

Twenty years of continuous productive scientific research performed jointly by two great nations is a unique milestone in the annals of medical history. The United States-Japan Cooperative Medical Science Program was wisely conceived in 1965 by President Johnson and Prime Minister Sato as a medium by which Japan and the United States might share their scientific affluence to benefit the underprivileged in Asia. Toward this end, plans were formulated with Doctors Toshio Kurokawa and Colin MacLeod in leadership roles. This culminated in the appointment of panels comprising competent scientists from each country and the formulation of indicated guidelines and investigative plans of study. Foremost with each step, including the financial commitment of funds, was the broad aim to develop new and scientific knowledge of practical importance to alleviate human misery through better health practices.

This volume is a concise review of two decades of scientific experiment and it details with sufficient coherence the achievements, new methods, new leads, and new directions which accrued during this exciting period.

Success in science is often not measured over a short term. Time is often an important yardstick of quality. Moreover, a considerable degree of wise perception is necessary to avoid diffuseness and blind roads. The Joint Committee and its panels of scientists have pursued many important leads, often to their fulfillment. Fitting examples are the remarkable new method of treatment of patients with Asiatic cholera by oral fluid replacement and better treatment for the ancient scourge of leprosy. There are many others.

More importantly, those responsible for the Program have recognized the likelihood of blind leads and have reshaped directions and guidelines in keeping with the development of new information and new technical opportunities. Study of the joint programs' 20-year experience reveals a flexibility, a trait so vitally needed if any long endeavor is to succeed.

Members of the delegations and joint panels acknowledge the encouragement and substantial support provided by the Governments of Japan and the United States.

Norio Suwa

Ivan L. Bennett, Jr.

In addition to the joint United States-Japan editorial effort which went into the drafting of this document, acknowledgement is made of our extensive collaboration in the preparation of this scientific report to the

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICES
DEPARTMENT OF HEALTH AND HUMAN SERVICES

Dr. Ivan L. Bennett, Jr.

Dr. Theodore E. Woodward

Dedication

Dr. Ivan L. Bennett, Jr., has been a member of the U.S. Delegation since 1967, and has served as Chairman since 1972. Under his excellent leadership, the scope of the program has expanded and its activities are full of remarkable achievements. His committment to this program has been unparalleled. Dr. Bennett has played an active and impressive role as the Chairman, and it is no exaggeration to say that he contributed a great deal to the accomplishments of this program.

Dr. Theodore E. Woodward is the only Delegation Committee member who has served from the beginning of the program, and he is most knowledgeable about the program. It may be fair to say that he is the encyclopedia of this program. He has participated in preparing all the communiques, reports, and four *Five Year Reports*. Through his warm personality and deep knowledge of medical science, he has worked as a major architect of the program.

On the occasion of the 20th Anniversary of the U.S.-Japan Cooperative Medical Science Program, we would like to dedicate this *Fourth Five Year Report* of the U.S.-Japan Cooperative Medical Science Program to Dr. Ivan L. Bennett, Jr., and Dr. Theodore E. Woodward.

Moreover, we sincerely wish that both doctors will continue in good health and will be able to contribute further to the improvement of this program designed to achieve better health for the people in developing nations as well as in our own countries.

Drs. Norio Suwa and Ivan L. Bennett, Jr.,
Chairmen respectively of the Japanese and
United States Delegations, U.S.-Japan
Cooperative Medical Science Program

MEMBERS OF THE JOINT COMMITTEE
U.S.-JAPAN COOPERATIVE MEDICAL SCIENCE PROGRAM

JAPANESE DELEGATION

Dr. Norio Suwa, *Chairman* (1979- ,
Member 1974-1979)
Professor Emeritus
Tohoku University
1-33, Kotake-cho, Nerima-ku
Tokyo 176, Japan

Dr. Hideo Fukumi (1977-)
Professor Emeritus
Nagasaki University
4-10-13, Seijyo, Setagaya-ku
Tokyo 157, Japan

Mr. Takakazu Kuriyama (1984-)
Director-General
American Affairs Bureau
Ministry of Foreign Affairs
2-2-1, Kasumigaseki, Chiyoda-ku
Tokyo 100, Japan

Dr. Wataru Mori (1982-)
Professor
Department of Pathology
Faculty of Medicine
Tokyo University
7-3-1, Hongo, Bunkyo-ku
Tokyo 113, Japan

Dr. Masumi Oike (1983-)
Director-General
Health Service Bureau
Ministry of Health and Welfare
1-2-2, Kasumigaseki, Chiyoda-ku
Tokyo 100, Japan

UNITED STATES DELEGATION

Dr. Ivan L. Bennett, Jr., *Chairman*
(1972- , Member 1967-1972)
Professor of Medicine
New York University Medical Center
550 First Avenue
New York, New York 10016

Dr. Barry Bloom (1981-)
Professor and Chairman
Department of Microbiology
Albert Einstein College of Medicine
1300 Morris Park Avenue
Bronx, New York 10461

Dr. Charles C.J. Carpenter, Jr. (1972-)
Professor and Chairman
Department of Medicine
Case Western Reserve University
Cleveland, Ohio 44106

Dr. Edward W. Hook, Jr. (1984-)
Professor and Chairman
Department of Internal Medicine
University of Virginia
School of Medicine
Charlottesville, Virginia 22903

Dr. David P. Rall (1984-)
Director
National Institute of Environmental
Health Sciences
P.O. Box 12233
Research Triangle Park, North Carolina 27709

JAPANESE DELEGATION — Continued

Dr. Manabu Sasa (1974-)
President
Toyama Medical and
Pharmaceutical University
2630 Sugitani
Toyama-shi 930-01, Japan

Dr. Tadao Shimao (1977-)
Director Emeritus
Research Institute of Tuberculosis
Japan Anti-Tuberculosis Association
3-1-24, Matsuyama, Kiyose-shi
Tokyo 204, Japan

Dr. Shiro Someya (1977-)
Advisor
Institute of Public Health
3-5-7, Naritanishi, Suginami-ku
Tokyo 166, Japan

Mr. Hiroshi Ueki (1982-)
Deputy Director-General
Science and International Affairs
Bureau
Ministry of Education, Science and Culture
3-2-2, Kasumigaseki, Chiyoda-ku
Tokyo 100, Japan

Dr. Yuichi Yamamura (1982-)
President
Osaka University
1-1, Yamadagaoka, Suita-shi
Osaka 565, Japan

Former Members

Mr. Shinichiro Asao (1980-1983)
Consul General
Consulate General of Japan
at New York

Dr. Hideo Katsuki (1978-1982)
Professor Emeritus
Chiba University
1-33, Yaoi-cho
Chiba-shi 280, Japan

UNITED STATES DELEGATION — Continued

Dr. Robert E. Shope (1977-)
Director
Yale Arbovirus Research Unit
Professor of Epidemiology
Yale University School of Medicine
New Haven, Connecticut 06510

Dr. G. Donald Whedon (1984-)
Consultant for Medical Research Programs
Shriner's Hospitals
International Shriner's Headquarters
2900 Rocky Point Road
Tampa, Florida 33607

Dr. Theodore E. Woodward (1965-)
Professor
Department of Medicine
University of Maryland
School of Medicine
Baltimore, Maryland 21201

Dr. James B. Wyngaarden (1982-)
Director
National Institutes of Health
Building 1, Room 124
Bethesda, Maryland 20205

Ex Officio

Mr. James Malone (1981-)
Assistant Secretary of State
Bureau of Oceans and International
Environmental and Scientific Affairs
Department of State
Washington, D.C. 20520

NIH Secretariat

Dr. Earl S. Beck (1970-)
Special Assistant to the Director, MIDP
NIAID and Coordinator of the U.S.-Japan
Cooperative Medical Science Program
National Institutes of Health
Bethesda, Maryland 20205

State Department Secretariat

Mr. William J. Walsh, III (1976-)
Coordinator for Biomedical Research and
Health Affairs
Bureau of Oceans and International
Environmental and Scientific Affairs
Department of State
Main State Building, Room 7820
Washington, D.C. 20520

Former Members

Dr. John Bryant (1979-1981)
Deputy Assistant Secretary for
International Health
Director, Officer of International Health
Department of Health and Human Services
Rockville, Maryland 20857

Former Japanese Members — Continued

Mr. Hiroshi Kitamura (1983–1984)
Director-General
Ministers Secretariat
Ministry of Foreign Affairs
2-2-1, Kasumigaseki, Chiyoda-ku
Tokyo 100, Japan

Dr. Daisuke Miura (1981–1983)
Commissioner, Environmental Disputes
Coordination Commission
1-6-1, Nagata-cho, Chiyoda-ku
Tokyo 100, Japan

Mr. Hitoshi Ohsaki (1979–1982)
Director-General
Bureau of Science and International
Affairs
Ministry of Education, Science and Culture
3-2-2, Kasumigaseki, Chiyoda-ku
Tokyo 100, Japan

Dr. Fujio Ohtani (1979–1981)
Director
Medical Care Facilities Financing
Corporation
2-2, 2-Ban-cho, Chiyoda-ku
Tokyo 102, Japan

Dr. Kenji Takeya (1979–1982), Deceased
Professor
Department of Medical Science
Kyushi University
2-1-1, Umade, Higashi-ku
Fukuoka 812, Japan

Former U.S. Members — Continued

Dr. Leighton E. Cluff (1971–1980)
Vice President
The Robert Wood Johnson Foundation
Forrestal Center
P.O. Box 2316
Princeton, New Jersey 08540

Dr. Donald Frederickson (1974–1981)
President
Howard Hughes Medical Institute
6615 Bradley Boulevard
Bethesda, Maryland 20817

Dr. Richard M. Krause (1979–1984)
Dean
Emory University
School of Medicine
Woodruff Medical Center
Administrative Building, Room 408
1440 Clifton Road NE.
Atlanta, Georgia 30322

Dr. James V. Neel (1971–1980)
Lee R. Dice University
Professor of Human Genetics
University of Michigan
School of Medicine
Ann Arbor, Michigan 48104

Mr. Thomas R. Pickering (1978–1981)
Ambassador to El Salvador
U.S. Embassy
San Salvador, El Salvador

Dr. Terrence A. Rogers (1978–1980)
Dean, John A. Burns School of Medicine
University of Hawaii at Manoa
Honolulu, Hawaii 96822

Dr. Nevin S. Scrimshaw (1974–1982)
Institute Professor
Department of Nutrition and Food Sciences
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

SECRETARIATS OF U.S.-JAPAN COOPERATIVE MEDICAL SCIENCE PROGRAM

Japan

Ministry of Foreign Affairs
  North American Affairs Bureau
  First North American Division
    Director, Yutaka Kawashima
    Deputy Director, Hitoshi Tanaka

Director
First North American Division
  Yutaka Kawashima

First North American Division
  Sachiro Nanami

Ministry of Health and Welfare
  Health Service Bureau

Director, Planning Division
  Hitomi Mori
Deputy Director, Masahiro Takamori
Deputy Director, Tetsuhiko Yoshida, M.D.

United States

National Institute of Allergy and
  Infectious Diseases (NIAID)

National Institutes of Health (NIH)

Director, NIAID
  Anthony S. Fauci, M.D. (1984)
  Richard M. Krause, M.D. (1975-1984)

Director, Microbiology and Infectious
  Diseases Program (MIDP)
  William S. Jordan, Jr., M.D.

Special Assistant to the Director, MIDP
  Earl S. Beck, Ph.D.

SECRETARIAT FOR LEPROSY AND TUBERCULOSIS PANELS

Japan

Tuberculosis and Intractable Diseases
  Division
Health Service Bureau
Ministry of Health and Welfare
  Director, Sotozo Kuboki, M.D.
  Officer, Masayoshi Kawai, M.D.

United States

Leprosy and Tuberculosis Program Officer
  Darrel D. Gwinn, Ph.D.

SECRETARIAT FOR CHOLERA PANELS

Japan

Office of Port Health Administration
Food Sanitation Division
Environmental Health Bureau
Ministry of Health and Welfare
  Director, Yoshikatsu Itakura
  Officer, Tsutomu Morikuni

United States

Cholera Program Officer
  Robert Edelman, M.D.
Assistant Cholera Program Officer
  Richard Horton, M.D.

SECRETARIAT FOR HEPATITIS PANELS

Japan

Infectious Diseases Surveillance
  Division, Health Service Bureau
Ministry of Health and Welfare
  Director, Sadahiko Nozaki, M.D.
  Officer, Shinsuke Morio, M.D.

United States

Hepatitis Program Officer
  Leslye D. Johnson, Ph.D.

SECRETARIAT FOR PARASITIC DISEASES PANELS

Japan

Infectious Diseases Surveillance
Division, Health Service Bureau
Ministry of Health and Welfare
Director, Sadahiko Nozaki, M.D.
Officer, Shinsuke Morio, M.D.

United States

Parasitic Diseases Program Officer
Harley G. Sheffield, Ph.D.

SECRETARIAT FOR VIRAL DISEASES PANELS

Japan

Infectious Diseases Surveillance
Division, Health Service Bureau
Ministry of Health and Welfare
Director, Sadahiko Nozaki, M.D.
Officer, Shinsuke Morio, M.D.

United States

Viral Diseases Program Officer
William P. Allen, Ph.D.

SECRETARIAT FOR IMMUNOLOGY BOARDS

Japan

International Science Division
Science and International Affairs
Bureau
Ministry of Education, Science and Culture
Director, Yoshikazu Hasegawa
Officer, Shinichi Ohta

United States

Immunology Board Program Officer
Bernard W. Janicki, Ph.D.

SECRETARIAT FOR ENVIRONMENTAL MUTAGENESIS AND CARCINOGENESIS PANELS

Japan

Planning Division
Health Service Bureau
Ministry of Health and Welfare
Director, Hitomi Mori
Officer, Tetsuhiko Yoshida, M.D.

United States

National Institute of Environmental
Health Sciences (NIEHS)

Director,
David P. Rall, M.D., Ph.D.

Environmental Diseases Program Officer
Michael D. Shelby

SECRETARIAT FOR MALNUTRITION PANELS

Japan

Health Promotion and Nutrition
Division, Health Service Bureau
Ministry of Health and Welfare
Director, Atsuaki Gunji, M.D.
Officer, Hiroyoshi Endo, M.D.

United States

National Institute of Arthritis,
Diabetes, and Digestive and Kidney
Diseases (NIADDK)

National Institutes of Health

Director, NIADDK
Mortimer B. Lipsett, M.D.

Malnutrition Program Officer
Van S. Hubbard, M.D.

DRAFTING COMMITTEE

Japan

Tadao Shimao, M.D.
Director Emeritus
Research Institute of Tuberculosis
Japan Anti-Tuberculosis Association
3-1-24, Matsuyama, Kiyose-shi
Tokyo 204, Japan

Shiro Someya, M.D.
Advisor
National Institute of Public Health
4-6-1, Shirokanedai, Minato-ku
Tokyo 108, Japan

Secretariat

Tetsuhiko Yoshida, M.D.
Deputy Director
Planning Division
Health Service Bureau
Ministry of Health and Welfare

United States

Theodore E. Woodward, M.D.
Professor
Department of Medicine
University of Maryland
School of Medicine
Baltimore, Maryland 21201

Earl S. Beck, Ph.D.
Special Assistant to the Director,
Microbiology and Infectious Diseases Program
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, Maryland 20205

Secretariat

Earl S. Beck, Ph.D.

BLANK PAGE

JOINT COMMUNIQUE
PRESIDENT JOHNSON AND PRIME MINISTER SATO
JANUARY 12 and 13, 1965

President Johnson and Prime Minister Sato met in Washington on January 12 and 13, 1965, to exchange views on the current international situation and matters of mutual interest to the United States and Japan. They were assisted by Secretary Rusk and Foreign Minister Shiina and Secretary-General Miki of the Liberal Democratic Party.

The President and the Prime Minister reviewed the present international situation and reaffirmed the partnership of the two countries which grows out of common beliefs and the shared objective of a lasting peace based on justice, freedom and prosperity for all people. They expressed a firm determination that the two countries should cooperate more closely in seeking this common objective. They agreed that for this purpose the two countries should maintain the closest contact and consultation not only on problems lying between them but on problems affecting Asia and the world in general.

The President and the Prime Minister, recognizing the valuable role of the United Nations in the maintenance of the peace and prosperity of the world, exchanged frank views on the difficult questions now confronting the United Nations, and agreed to continue cooperative efforts to strengthen the functions of the United Nations and to enhance its authority.

. . . .

The President and the Prime Minister, mindful of the many areas of human health which are of great concern to all the peoples of Asia, agreed to undertake a greatly expanded program of cooperation in medical science with respect to such diseases as malaria, cholera, schistosomiasis, tuberculosis, and stomach cancer, in addition to cooperative efforts on problems of air pollution and pesticides. As a first step to implement the agreement, they agreed to convene a conference of the foremost medical scientists from the United States and Japan to work out the details of the new program for discussion with other governments concerned.

The President and the Prime Minister expressed their satisfaction with the meeting just held and their desire to continue to maintain close personal contact.

ABOVE: Inaugural meeting of the U.S.-Japan Cooperative Medical Science Program held in Tokyo, April 1965. Foreground, left to right—Dr. Kurokawa (Chairman), Mr. Yasukawa, Mr. Okano. At table, left to right—Drs. Woodward, Watt, Audy, Francis, MacLeod (deceased, Chairman), Shannon, Bennett, Stanley. **RIGHT:** Former Co-Chairmen of the Joint Committee, Dr. Colin MacLeod and Dr. Toshio Kurokawa (right), discussing an agenda item prior to the July 29-30, 1971 meeting. **BELOW:** The opening session of the 12th Joint Committee Meeting held at the Ministry of Foreign Affairs in Tokyo, September 24-25, 1976.

ABOVE LEFT: Minister Nakayama, Japanese Government representative; Dr. Toshio Kurokawa, Chairman of the Japanese Delegation; Dr. Ivan L. Bennett, Jr., Chairman of the U.S. Delegation; and Mr. Justin Bloom, U.S. Government representative, at the 12th Joint Committee Meeting. **ABOVE RIGHT:** The 7th Joint Committee Meeting on July 29-30, 1971, in Bethesda, from left to right: back row—Mr. Pollack, Dr. Cluff, Dr. Woodward, Mr. Inumaru, Dr. Iwasaki, Mr. Nomura; middle row—Drs. Neel, Ivan Bennett, Jr., Lennette, Marston, H. Stanley Bennett, Yanagisawa, Takizawa; front row—Drs. DuVal, MacLeod, Kurokawa, Soda. **BELOW LEFT:** Drs. Sasa, Someya, Fukumi, and Suwa, attending a Subcommittee meeting in Bethesda on July 25, 1979. **BELOW RIGHT:** Drs. Jordan and John Seal (deceased) at the July 26, 1979 Joint Committee Meeting in Bethesda.

ABOVE: Members of the Viral Diseases Panels at the Joint Meeting in Oiso, Japan, September 9–11, 1984, from left to right—U.S. members (left) Drs. William Allen, Thomas Yuill, Phillip Russell, Albert Kapikian, and Neal Nathanson. Japanese members (right) Drs. Nobuo Hashimoto, Akira Oya, Konosuke Fukai, Tasuke Konno, Akihiko Kawai.

LEFT: Joint Parasitic Diseases Panel Meeting at Unzen, Nagasaki, August 1974.

ABOVE LEFT: left to right, Drs. Norio Suwa, Toshio Kurokawa, Shiro Someya, Hideo Fukumi, Sotoza Kutsuki, and Mr. Haruhiko Tanaka in Honolulu attending a February 1978 Subcommittee Meeting. ABOVE RIGHT: Japanese and U.S. participants enjoying the hospitality of Dr. and Mrs. Theodore Woodward on Parsons Island, on the occasion of the 17th Joint Committee Meeting. BELOW: 17th Joint Leprosy Panel Meeting held at NIH, Bethesda, Maryland, July 1981.

Introduction

This is the fourth in a series of reports for the U.S.-Japan Cooperative Medical Science Program (U.S.-Japan CMSP). These reports cover 5-year periods of Program events and scientific progress. The *First Five-Year Report, 1965–1970*, was adopted by the Joint Committee at its sixth meeting. The *Second Five-Year Report, 1970–1975*, was accepted by the Joint Committee at its 11th meeting. This publication was dedicated to Dr. Colin MacLeod, Chairman of the U.S. Delegation and Co-Chairman of the Joint Committee from 1965 until his death in 1972. The *Third Five-Year Report, 1975–1980*, was adopted by the Joint Committee at its 16th meeting. This report was dedicated to Dr. Toshio Kurokawa, Chairman of the Japanese Delegation and Co-Chairman of the Joint Committee from 1965 until his retirement in 1979. The *Fourth Five-Year Report, 1980–1985*, is dedicated to Dr. Ivan L. Bennett, Jr., Chairman of the U.S. Delegation and Co-Chairman of the Joint Committee, and to Dr. Theodore E. Woodward, member of the Delegation and of the Joint Committee since its inception in 1965.

Preliminary discussions concerning the preparation of a *Fourth Five-Year Report, 1980–1985*, of the U.S.-Japan CMSP were held at the 19th Joint Committee meeting but it was not until the 20th meeting that the project was officially launched with a publication date set for July 1985. This fourth report is considered special because it represents two decades of continuous collaborative effort and scientific exchange between scientists of Japan and the United States. Noteworthy is that the Program continues to gain momentum with the passing years.

The Joint Committee agreed that the same general format used for previous reports be adopted and that the U.S. Delegation accept the responsibility for designing the cover, a responsibility that alternates between the two countries. The Joint Committee accepted the recommendation of the Subcommittee that "The Historical Review of Program Guidelines, 1965–85" be included in this report.

ORGANIZATION AND HISTORY

In January 1965, the President of the United States and the Prime Minister of Japan met in Washington, D.C., to discuss many important matters concerning the two nations. It was during this meeting that the two heads of state agreed to undertake a greatly expanded, joint cooperative research effort in biomedical sciences, concentrating on health problems of recognized importance in Asia. The new bilateral program was named the United States-Japan Cooperative Medical Science Program (U.S.-Japan CMSP).

Medical advisers from the respective countries met in April and again in October of 1965, selecting cholera, leprosy, parasitic diseases, tuberculosis, and viral diseases as initial areas for collaborative research. In 1966, research on malnutrition was added, and 6 years later, in 1972, the Program was broadened to include investigations on environmental mutagenesis and carcinogenesis. In 1979, research on viral hepatitis, a disease of worldwide importance, was added, expanding the program from seven to eight disease areas. This was not to be the last addition because the Program continued to gain momentum and grow. In 1981, a decision was made to include immunology as a new research area, since it had direct application to most, if not all, the other eight areas.

The Program was defined to operate within a bilateral governmental framework which could involve scientists and facilities in Third World countries in collaborative efforts of mutual interest. Cooperation with the World Health Organization (WHO) has always been considered appropriate and has been encouraged.

The relevant regions in Asia are not specifically delineated but are generally understood to include the Republic of Korea in the north, India and Pakistan to the west, and other intervening nations in the broad Pacific basin.

The United States and Japan each support the costs of its own scientific projects and meetings. Research by U.S. scientists is funded by the United States Government and that of Japanese scientists by the Japanese Government.

The Joint Committee is expected to advise its respective governments on the scope, direction, and other broad aspects of the program and to develop review procedures necessary to assure fulfillment of the objectives for which the program was established.

The panels have achieved significant progress in their respective areas of research. To keep the research focused, each panel developed specific guidelines which have been appropriately refined and modified as research progressed. The results of the three previous publications are briefly summarized in this current report.

The fourth 5 years of the Program saw further progress through increased participation of scientists in collaborative research and through successful conferences which provided a forum for active discussion, careful analysis of data, and mutual stimulation of the total research effort.

ACTIVITIES OF THE JOINT COMMITTEE

During the fourth 5-year period, Joint Committee meetings continued to be held annually, alternating between the United States and Japan. The meetings of the Joint Subcommittee on Program Review and Planning were held in Hawaii each February and again just prior to the Joint Committee meetings.

In 1980, the 16th Joint Committee meeting was held in Tokyo. A *Third Five-Year Report, 1975–1980*, describing the scientific progress and administrative activities of the U.S.-Japan Program, was adopted and dedicated to the former Chairman of the Japanese Delegation and Co-Chairman of the Joint Committee, Dr. Toshio Kurokawa, who was present to receive this special tribute from his colleagues. Preceding the Joint Committee meeting, a very successful Immunology Symposium was held. The Joint Committee was pleased with the content of the symposium, and enthusiastically recommended that the organizers prepare research guidelines as a preliminary step for including immunology as a new research area in the U.S.-Japan Program.

New initiatives under the Japan-United States Non-Energy Research Cooperative Agreement were discussed by the Joint Committee, particularly vaccine development and research on interferon and other antiviral substances. It was the consensus that it was unnecessary to create special panels for vaccine development, and little enthusiasm was expressed for vaccines lying outside programmatic interests. The development and production of chemical antivirals was not felt to warrant immediate action, since pharmaceutical companies were already involved in this area. However, it is considered important that scientists in the two countries maintain a dialog about developments in these areas. An interest in interferon is to be explored sometime in the future.

The 17th Joint Committee meeting was held in Bethesda on July 16 and 17, 1981. Just prior to this meeting, the Leprosy and Parasitic Diseases Panels held informative conferences. The joint conferences initiated a year of formal review that was completed at the 18th Joint Committee meeting. Deliberations on the relationship of interferon to the U.S.-Japan program were completed. The Committee decided that a new program on interferon not be formed, although an agreement was made that exchanging information and keeping the Committee informed of new developments was the responsibility of all panels, but especially the Hepatitis and Viral Diseases Panels.

The Joint Committee enthusiastically approved the Immunology Board as a new addition to the U.S.-Japan Cooperative Medical Science Program, increasing the number of program areas from eight to nine. The categorical designation of "Panel" was replaced by "Board" to indicate a discipline- rather than a disease-oriented research emphasis. Support for the Board was to be provided by the Ministry of Education rather than the Ministry of Health and Welfare.

The Joint Committee initiated discussions on possible options for reducing Program-associated costs without compromising the quality of scientific research. The fiscal difficulties appeared to be more acutely related to the United States than to Japan. This topic was referred to the Subcommittee on Program Review and Planning for additional discussion and for recommendations to be presented at the 18th Joint Committee meeting.

The review criteria developed in 1971 by the Subcommittee for panel review and evaluation were modified. As originally designed, six questions required written answers by the reviewers. The sixth and last concerned the final disposition of the panel. The Committee decided that this placed a responsibility on the reviewers that ought to be borne by the Committee. Consequently, the review criteria were modified to reflect this decision.

In 1982, the 18th Joint Committee met in Tokyo on July 22 and 23. The reviews of the Leprosy and Parasitic Diseases Panels were completed with the reviewers recommending that these panels be continued. It was at this meeting that the Joint Committee made the decision that henceforth the Leprosy and Tuberculosis Panels would hold combined meetings. Since the two panels shared areas of mutual interest, it was felt that such a realignment would strengthen both programs. Each panel was to maintain its identity as a distinct and separate entity, but common mycobacterial research interests were to be pursued jointly. Also, the Joint Committee recommended that the Joint Parasitic Diseases Panels plan a symposium to review the state-of-the-art of parasitic diseases research prior to an in-depth review of the panels' research guidelines.

The 19th Meeting of the Joint Committee of the U.S.-Japan CMSP was held at the National Institutes of Health in Bethesda, Maryland on July 21 and 22, 1983. The 3 days prior to this meeting, the Joint Parasitic Diseases Panels held a very successful "Symposium on Current and Unresolved Problems in Parasitic Diseases." Immediately following the symposium, the panels prepared revised guidelines and submitted them for consideration by the Joint Committee who in turn referred them to the Subcommittee on Program Review and Planning for thorough review and evaluation. These guidelines were an agenda item at the 20th Joint Committee Meeting.

The dialog for reducing Program costs was continued from the last Joint Committee meeting. After considering several alternatives, the Committee recommended that whenever possible the annual joint panel and board conferences be held in conjunction with large domestic or international meetings in an attempt to conserve travel funds, since a number of the involved scientists would, in all likelihood, already have received travel support. Joint conferences or panel meetings were to be held in association with important international meetings provided this arrangement did not disrupt the budgetary allocations of either Japan or the United States or compromise program continuity.

The review reports of the Joint Panels on Tuberculosis and Viral Diseases were accepted by the Joint Committee who recommended that these panels be continued.

In 1984, the 20th Meeting of the Joint Committee was held in Tokyo on July 19 and 20. Since no joint scientific conferences were planned for the first 2 days of the week, the Japanese Delegation hosted a field trip to Tsukuba City (Science City) for the U.S. Delegation and invited guests. Both Delegations agreed that the visit was very informative, since much had been learned about the educational concepts of the University and of applications of high energy physics to biomedicine, especially for improved cancer diagnosis and therapy.

Since the Joint Hepatitis and Environmental Panels had held scientific conferences in conjunction with other meetings, the morning of Wednesday, July 18, was set aside for the Chairmen and selected colleagues to present scientific progress since their last formal review. The presentations were held simultaneously in separate rooms in the new building of the Ministry of Health and Welfare. The sessions were well attended by Delegation members and other interested members of the Japanese scientific community. The Joint Committee was pleased with the arrangement and agreed that such a format provided the Program with an additional degree of flexibility. These presentations initiated a year of formal review for the two panels.

The expanded guidelines for the Joint Parasitic Diseases Panels were accepted as recommended by the Joint Subcommittee on Program Review and Planning. Although the workscope was expanded, the language used was sufficiently selective to prevent diffuseness.

At this meeting, the Joint Committee suggested that the Joint Malnutrition Panels plan a symposium to review current research in the field of nutrition. After the symposium, the guidelines will be evaluated in terms of technological development and new research opportunities. The symposium will initiate a year of formal review for these panels.

Plans were made for organizing and publishing the *Fourth Five-Year Report, 1980–1985*. This report represents two decades of scientific achievement by the U.S.-Japan CMSP.

GUIDELINE CHANGES, 1965–85

Preface

The prevalance of human misery caused in large part by widespread illness prompted President Lyndon Johnson and Prime Minister Eisaku Sato to propose a cooperative Medical Science Program whose goal would be to improve the health of the people of Asia. Acting promptly and with objective precision, the two senior science advisers, Colin MacLeod, M.D. and Toshio Kurokawa, M.D., carefully and wisely recruited leading biomedical scientists from the United States and Japan to plan the program. Through working conferences and seminars, they formulated plans and established practical guidelines which culminated in the evolution of a workable and practical medical science program. A measure of its success is this report, which marks the end of two decades of joint scientific endeavor between two nations whose goal is to assist in the welfare of others. The current Co-Chairmen, Dr. Ivan L. Bennett, Jr. and Dr. Norio Suwa, not only continue the established traditions but through their wise guidance have sharpened focus in some areas and broadened the scope in others.

Honorable Edwin O. Reischauer, the U.S. Ambassador to Japan, received the eight U.S. Delegation members in 1965 at the Embassy in Tokyo, and during the briefing remarked that timing for initiation of the Joint Program seemed to be unfortunate because of the current sensitivity and unrest in Japan caused by the Vietnam experience. A few hours later the United States delegates met with their Japanese counterparts, for the first time, at the National Education Hall.

In his opening remarks, Dr. Kurokawa likewise commented that matters had changed since President Johnson and Prime Minister Sato had proposed the initiation of a Joint Medical Science Program. In spite of these concerns, these reservations were not considered as a deterrent to members of the respective Delegations. The charter group met frequently and held objective and open-ended discussions which resulted in a program which has become a model of cooperative medical research between two major nations. Credit is due to the dedicated, imaginative, and capable persons of this founding group who planned the goals and guidelines, recruited the key scientific personnel, and helped acquire the resources necessary to achieve the desired objectives.

In 1965, the approaches to take were uncharted and unclear simply because the magnitude of health problems exceeded the availability of resources as well as the possible limitations to recruit competent scientists with ability and adequate time to contribute. To these aims, after much discussion, sifting of reliable epidemiologic data, and evaluation of the feasibility of success, the joint delegation established five panels. Panel Chairmen received guidance and logistic support and were charged with further recruitment of working scientists. They were expected to formulate defined standards and objectives within the guidelines, subject to approval of the Joint Committee.

The initial disciplines chosen for study, in the form of panels, were cholera, leprosy, tuberculosis, and parasitic and viral diseases.

Cholera was a natural study choice because of its wide Asian distribution and its very significant contribution to high mortality. Neither its pathogenesis, means of prevention, or treatment were understood, although inroads into these problems had been initiated by other agencies such as the U.S. Navy Research Unit in Taiwan, and the Southeast Asia Treaty Organization Laboratory in Dacca managed by the U.S. National Institutes of Health. There was good reason to expect additional emphasis and ultimate success under aegis of the U.S.-Japan Cooperative Program.

In spite of similar limitations of scientific data regarding tuberculosis and leprosy, it was patently apparent that new information in pathogenic mechanisms, characterization and purification of the respective antigens, and better chemotherapeutic and prophylactic techniques were required if tuberculosis and leprosy were ever to come under better control.

To avoid diffuseness and to assure effectiveness, the program in parasitic diseases was purposely limited to the study of schistosomiasis and filariasis. These two diseases afflict millions of children and adults in Asia. Gaps were obvious in the understanding of the fundamental technical information needed to detect, treat, and prevent them. Malaria was excluded for study simply because other agencies, including military agencies, were already intensively evaluating the problem.

Difficult months were ahead for the Viral Diseases Panels, not related to a limit of study categories. The U.S. panel soon initiated work on rabies, smallpox vaccine, dengue, and other arbovirus infections. The Japanese panel, after unavoidable delays in organization, finally came into focus and picked up lost time.

Japanese authorities could not have better accomplished their organizational planning during this early period as was evidenced by their careful choice of members to the delegation and panels. The same may be said for the United States.

This is a brief historic description of those events which culminated in the U.S.-Japan Cooperative Medical Science Program. To follow is a documented account of the indicated and relevant changes in guidelines and direction which typify the flexibility rather than unbending rigidity that the Joint Delegations have pursued in quest of their common objectives.

Purpose

A review of the guidelines of the U.S.-Japan CMSP was undertaken upon the request of the Joint Committee to show how the panels' guidelines evolved and how they have changed from 1965 to 1985.

Background

Considerable thought and effort was expended in developing appropriate guidelines for each of the panels. In addition, it was recognized that if collaborative research were to evolve it would be necessary to have available in both countries sufficient staff, ample funding, and necessary research materials or specimens to pursue the stated research objectives. It was understood that scientists in both countries would have special research interests, and this was considered a strength, provided the research effort was complimentary to the overall program effort. Exchanges of reagents, research materials, and personnel were encouraged. A great deal of emphasis was placed on developing an active fellowship exchange program. Funding was provided for the Japanese Delegation, and scientists traveled from Japan to work in U.S. laboratories. Unfortunately, funds were never provided by the U.S. Delegation and a fellowship program was never formally established. In spite of the lack of specific funding, an informal exchange of scientists is still encouraged by both sides, with more Japanese scientists participating than Americans, but the formal guidelines were eventually modified and all references to a fellowship exchange program were omitted. Annual joint meetings of panels were to be held, alternating between Japan and the United States. These meetings served as the mechanism for developing collaborative programs.

The guidelines were and still are the basis for including research projects in the U.S.-Japan Program, and are the basis for developing conferences, symposia, and workshops. Guidelines changes approved by the Joint Committee may alter program emphasis or content.

In 1970, the Joint Committee established a Subcommittee on Program Review and Planning which meets twice yearly and writes criteria for the formal evaluation of each panel once every 5 years. The intent of the guidelines was really the linchpin for developing the review criteria.

Origin of the Program

The U.S.-Japan CMSP was the result of a meeting between Prime Minister Sato and President Johnson in Washington, D.C., on January 13, 1965, at which time the two heads of state decided to undertake a cooperative medical science program for improving the health of the people of Asia. To implement this cooperative agreement, a Joint Planning Committee composed of prominent scientists from both countries met in Tokyo April 19–21, 1965, to develop the organizational structure of this new program. The diseases selected for study were cholera, leprosy, parasitic diseases (schistosomiasis and filariasis), tuberculosis, and virus diseases. Panels for each of the diseases or disease categories were to be appointed prior to the October 4–7, 1965, Honolulu conference. Although malnutrition was recognized as an important area for consideration, it was felt that additional information was needed before adding it to the program, so an *ad hoc* planning committee was appointed to develop a study plan.

Development of Research Goals and Objectives

The Joint Committee and five panels met for a conference in Honolulu October 4–7, 1965, to review the status of each disease and to develop appropriate research objectives and goals for each of the five panels. **These are presented as recorded in the final report of that meeting:**

Cholera Panel

The members of the Cholera Panel unanimously propose that an interchange of Japanese and Americans engaged in cholera research be initiated in the immediate future, that the possibility of establishing new locations for collaborative field work be thoroughly explored, and that the following specific research programs be vigorously pursued:

1. Notwithstanding the recognition of the role of sanitation in the ultimate eradication of this disease, the highest priority in the overall program should be assigned to the development of an effective vaccine which consistently confers long-term immunity against cholera. Major emphasis must be placed on the improvement of whole-cell vaccines, with studies directed toward development of effective adjuvants, and the selection of strains and cultural conditions for maximum antigen production. Further efforts will be directed toward the isolation and purification of protective antigens. Studies must be initiated to determine which antibodies confer immunity in the laboratory model as well as in humans. Efforts will also be made, in collaboration with the World Health Organization, to standardize methods used in the assay of vaccines.

2. Immediate full-scale efforts must be directed toward development of more effective treatment of pediatric cholera patients and toward demonstrating the effectiveness and practicality of a single standard intravenous electrolyte replacement regimen in treating large numbers of patients in epidemic situations. In this respect, immediate steps must also be taken to develop a laboratory model in a large animal (e.g., the monkey or chimpanzee) in which a disease closely simulating human cholera can be consistently established.

3. The establishment of a cholera surveillance system, including collection of appropriate specimens throughout Asia, should be undertaken in collaboration with the World Health Organization. This system should monitor bacterial resistance patterns as well as serotypes and bacterial phage types. Efforts directed toward development of better methods to identify the carrier state will include further evaluation of phage techniques and search for spheroplast forms of *V. cholerae* in convalescent cholera patients.

4. Bacteriologic investigations should include further viability studies, evaluation of new selective media, studies of the role of noncholera vibrios in disease, and studies of bacterial genetics including drug resistance patterns.

5. Studies of pathogenesis of cholera should be directed toward determining which toxin(s) cause the pathophysiologic disturbances in the cholera patient, the mechanism of action of the toxin(s), and the chemical characterization and immunologic properties of such toxin(s).

6. Additional clinical studies should be designed to establish the minimal effective therapeutic dose and route of administration of two or more antimicrobial agents and to find an economical method of prophylaxis effective in epidemic situations.

The widespread dissemination of cholera during the past 5 years, and the unnecessarily high fatality rate in patients affected by this disease, emphasize the urgency of the implementation of the above proposals. It is the strong feeling of the Joint Cholera Panel that the vigorous and integrated efforts of the U.S.-Japan CMSP will succeed in achieving its two major goals: the immediate goal of making effective therapy available to cholera patients throughout Asia, and the long range but ultimately more important goal of developing an effective vaccine which consistently confers long-term immunity against cholera.

Leprosy Panel

The U.S.-Japan Panel on Leprosy devoted its attention to a discussion of the leprosy research that needs to be undertaken as an assistance to those regions of Asia where the disease exists as a problem of great public health importance. The joint panel found itself in complete agreement on the measures to be undertaken, and in its sessions enjoyed discussion and exchange of information of great benefit.

It agreed that the following scientific areas deserve special attention:

1. **Cultivation**. The inability to grow *Mycobacterium leprae in vitro* is a major impediment to research progress. This topic is already receiving major attention in the two countries, but increased efforts are indicated. Attempts should be directed toward growth in tissue culture, and in an artificial medium.

2. **Animal transmission**.

a. An experimental system for the growth of *M. leprae*, the mouse foot pad system, is currently available, and it should be introduced into more research laboratories, especially to assist the other work described here. Improvements in the system should be sought in increased bacillary growth, both locally and systemically. Increased yield would supply bacilli for other research.

b. Other animal models should be sought, since they might also meet these same needs. A systemic infection might provide a more complete reproduction of systemic human disease.

3. **Drugs against leprosy**. The drugs available at present, though eventually effective in nearly all cases, must be administered for many years, and lepra reactions occur in many patients.

a. The currently available experimental system should be utilized by more laboratories to search for new drugs. We would hope to interest pharmaceutical firms in the search also.

b. There is need for increased clinical testing of new drugs and of modification involving older drugs, especially sulfones.

c. Increased participation by pharmacologically trained scientists is needed for these efforts.

d. Investigation into the mechanism of lepra reactions and drugs to prevent them also are needed.

4. **Chemoprophylaxis** has great potential value as a method of leprosy control in developing countries. It is necessary to learn more about the minimal effective blood and tissue levels of such drugs as DDS, and how these might be achieved with present preparations and modifications thereof. If the results are favorable, field trials would be indicated in the future.

5. **Vaccination** is also of great potential value as a control method in developing countries. BCG has prophylactic activity against *M. leprae* in mice, and experience suggests that it is effective in humans also. Field trials are now being carried out by third countries and their results are expected soon. When these are available, it may be indicated for us to consider further field trials of vaccine.

6. **Antigens similar to lepromin and mechanism of lepromin reactions**. The lepromin test has been used as a method to measure the responsiveness of the human body to *M. leprae*. However, the collection of materials for the production of lepromin is becoming increasingly difficult. We must consider research on substitutes for lepromin by antigens of similar quality. The immunological significance of lepromin reactivity is not yet clarified, and studies should be undertaken in this area.

The two panels also agreed that the following general supportive measures should also be adopted, in order to attract new research talent into leprosy research and to increase the productivity of those already working in the field.

1. Post-doctoral fellowships for research specifically on leprosy in research laboratories and in leprosy-endemic areas.
2. Grants for short-term visits of a few months to endemic areas by younger research scientists who express genuine interest for working experience with leprosy.
3. Grants for short-term visits to endemic areas by more mature scientists to encourage them to undertake research on leprosy.

> Items (2) and (3) are stimulated by past difficulties in providing opportunities for scientists to gain first-hand experience with the disease.

4. A supply of clinical materials, especially skin biopsies, for qualified scientists in our countries. Present research efforts, especially in cultivation, are very seriously thwarted by insufficient supplies of this material.
5. In Japan, a General Meeting on Leprosy has been held annually for many years. It is hoped to establish an annual research conference on leprosy in the United States. Arrangements should be made to enable U.S. and Japanese scientists to participate significantly in one another's conferences. In addition, joint U.S.-Japan conferences should be held whenever possible.

Parasitic Diseases Panel

Schistosomiasis and filariasis are diseases that afflict hundreds of millions of people and cause untold human suffering and economic loss. They inhibit the development of emerging nations. They must be eradicated.

The combined U.S.-Japan Panel on Parasitic Diseases has come to an agreement as to which research areas should be fostered to develop effective measures of control and eradication of these two helminthic infections. The panel has also agreed on various means by which its recommendations for enhanced research may be fulfilled.

The research areas recommended include very broad problems: It is necessary to continue study of disease transmission in relation to the natural environment, and it is essential also that experimental laboratory approaches be exploited. These statements apply both for schistosomiasis and filariasis.

Somewhat more specifically, the U.S. and Japanese panels have pooled their ideas on the relative importance of various research approaches. For schistosomiasis, it is believed that work is necessary on the following subjects: biology and biochemistry of the parasite and its vectors, especially in relation to the differences which exist between *Schistosoma japonicum* and other human schistosomes, and which may account for differences in pathogenicity and susceptibility to drugs; chemotherapy and chemoprophylaxis, immunology and serology; pathology and clinical evaluation; and ecology and epidemiology. For filariasis, the items of high priority are the study of the mechanism of action and the pharmacology of antifilarial drugs; the biology and biochemistry of the vector and parasite; and immunology and serology; redefinition of the clinical disease, its pathogenesis and pathology; and epidemiology and ecology.

The importance of these research areas obviously compels additional support. Each country has special interests, experiences, and qualifications, and undoubtedly the efforts which will evolve in each country will depend on the labor resources and the material available for study. The panel recommends also that collaborative research programs be developed that will allow for the most effective utilization of combined Japanese and American conceptual and technical abilities applicable to the large task at hand.

In this connection, it is recommended that the exchange of scientific personnel between the two countries be enhanced. The exchanges should be for research training and for the prosecution of research. They would allow for training in laboratory and field techniques in both countries, and for the utilization by experienced investigators of the research material now available.

In addition to exchange of personnel, it is important that exchange of research materials and information be facilitated. Laboratory investigations in both countries could be encouraged by the exchange of parasite and vector species. The exchange of information should be organized to include listings of laboratories and workers and their special research interest in both countries, and data on special training and research facilities. Furthermore, a scientific newsletter issued at regular intervals, perhaps twice a year, would provide for better awareness on the part of each national group of the research progress of the other.

Finally, the combined panel believes that it would be very helpful to sponsor conferences on special research problems, bringing into the discussion third-country participants and specialists from other disciplines. A propitious time for a first conference would be just prior to the Pacific Science Congress, August 1966. The combined panel has agreed to choose the topic for discussion from among the following: comparative physiology and biochemistry of *Schistosoma mansoni* and *S. japonicum*; pharmacology and mode of action of selected drugs against schistosomiasis and filariasis, particularly diethylcarbamazine and an anti-schistosomal nitrothiazole compound; evaluation of present immuno-diagnostic tests for schistosomiasis and filariasis in relation to (a) development and presence of functional immunity and (b) determination of therapeutic efficiency of drugs; or pathological and clinical studies on filariasis and schistosomiasis.

The combined panel submits the above recommendations to the U.S.-Japan Committee with a sense of urgency. It is indeed time that a concerted attack be made on schistosomiasis and filariasis. It remains only for us to thank the Committee for this opportunity to present the needs for work on these diseases and to develop a program that will have a significant impact on them.

Tuberculosis Panel

The Joint Tuberculosis Panel of the U.S.-Japan Cooperative Medical Science Program recognizes that all areas of tuberculosis research are important and require strengthening. Under this joint program, however, the greatest practical achievements can be attained by the expansion and coordination of research in the field of tuberculosis immunology.

The program recommended involves the utilization of new technology and new knowledge developed in the general fields of biochemistry and immunology to solve specific problems in tuberculosis immunity and allergy. This work will bring about an increased understanding of the mechanisms of immunity and hypersensitivity in tuberculosis and the development and testing of new potential agents for immunization against tuberculosis.

Under this program, each government should support the necessary expansion of research facilities, research activities, and training of scientists. This will involve establishment of new laboratories, modernization of existing facilities, and training of both new and established scientists in the use of new tools and techniques.

Greater collaboration between American and Japanese scientists is essential to the implementation of this program. This collaboration should basically and promptly take three forms:

1. The first is the establishment of better means of written communication between Japanese and Americans working in the field of experimental tuberculosis.
2. The holding of joint conferences in Japan and in the United States where the scientists of these and other countries can meet and exchange new, first-hand information in both a formal and an informal manner. The first conference should be held in Japan in the spring of 1966 in conjunction with the U.S.-Japan Cooperative Research Conference on Leprosy.
3. The facilitation of the exchange of working scientists between cooperating laboratories in Japan and in the United States.

Virus Diseases Panel

The joint deliberations of the United States and Japanese Panels on Virus Diseases resulted in a consensus that research and investigations on virus diseases at this stage of planning should be focused on two major areas, viz.: the arthropod-borne viruses and the respiratory viruses.

Because of the limitations of talent, labor, and finances, it becomes necessary to circumscribe the areas at which research should be directed. In consequence, six arboviruses are regarded as of primary importance. These are:

1. Japanese encephalitis
2. The dengue viruses
3. Chikungunya
4. Kyasanur Forest disease virus
5. Murray Valley encephalitis virus
6. West Nile virus

However, it would seem desirable for the immediate future to concentrate on two, namely Japanese encephalitis virus and the dengue complex.

With regard to Japanese encephalitis, it is a joint panel recommendation that studies directed at strain differences and at immunity and prevention have a priority position. It is important to determine whether actual strain differences occur, both with respect to differences that arise over a temporal span and as a reflection of geographical distributions. The importance of such differences lies not only in their representing differences in the reservoir host and in reservoir-vector relationships, but also in determining which strains are important for inclusion in vaccines. The panel also recommends that studies be conducted on the pathogenesis of Japanese encephalitis and on the relation of immunity to reinfection. Additional studies are highly desirable on the ecology of reservoir hosts, with special attention directed at the situation that exists in interepidemic periods. Last, it appears cogent to determine if minor infections, and even inapparent infections, with Japanese encephalitis virus give rise to damage of the central nervous system, with subsequent appearance of psychiatric problems and mental retardation. Therefore, it is recommended that long-term followup studies with psychometric measurements be conducted on children suffering mild or subclinical infections.

In the dengue complex of viruses, studies should be directed to the resolution of what underlies so-called "hemorrhagic fever" in Southeast Asia; i.e., is this phenomenon a reflection of differences in virulence of dengue virus strains, or of differences in the genetic background of the host? Along these lines, information is needed as to whether preexisting antibody to other Group B arboviruses predisposes to serious complications consequent upon a dengue virus infection. Studies are also needed to determine the extent, if any, to which infection with one Group B arbovirus confers protection against infection with another. The large epidemics of dengue which have occurred at frequent intervals point to the need for vaccines, and these are mentioned further below.

As concerns the respiratory viruses, primary attention should be given to the influenza viruses. It is desirable to determine whether Asia gives rise to new strains and what the reservoir of the virus might be in this area. Studies are also desirable to determine whether influenza viruses are transmissible between human and animals in a human environment; as an example, the relationship of equine influenza to human influenza. Studies on virulence and variation of influenza viruses are deemed necessary to a better understanding of the epidemiology and immunology of the disease. It is also recommended that physiologic studies be conducted on the pneumonitis produced by influenza viruses. It is necessary also that studies be continued and expanded with respect to influenza virus vaccines.

The parainfluenza viruses are of unquestioned importance, but a knowledge is needed of the incidence or prevalence of infections with these viruses in children in Asia. It is recommended that information be obtained on this point as a requisite for future studies. It is also recommended that, when studies on nutrition are undertaken in various areas, studies on respiratory and other viral diseases be undertaken simultaneously to determine whether these diseases present the same or different manifestations in malnourished populations.

On the matter of adenoviruses, the joint panel recommends at this time only that new culture systems be sought that will eliminate contamination of adenoviruses with adventitious viruses. This will greatly increase the safety margin, with respect to oncogenicity, of adenoviruses used for vaccines. In the matter of RS, or respiratory syncytial viruses, the panel recognizes the difficulties that have been associated with the production of a vaccine and recommends that studies be expanded toward development of a vaccine utilizing purified viral fractions.

Vaccines have been mentioned in relation to the arboviruses as well as the respiratory viruses. The development of such vaccines rests on a number of common factors, among which might be mentioned the desirability of fractionating viral preparations and studying such fractions for their antigenic capacity and protective properties. By analogy with what is known about a number of viruses, it would seem likely that immunogenic fractions can be separated out.

Such fractions would be useful in the preparation of vaccines from various arboviruses and studied from the standpoint that sequential immunization with a series of different viruses would lead to a broad protection. Consideration must also be given, however, to the postulated possibility that a dengue virus infection in a child with pre-existing antibody to other Group B viruses may give rise to a massive anaphylactoid reaction.

Although much work has been done on influenza vaccines, additional studies are recommended on the use of multiple antigen vaccines; i.e., vaccines containing active antigenic fractions from different and distinct viral strains. Comprehensive inquiries should also be directed at the usefulness and effectiveness of adjuvants, whatever their nature, in stimulating high levels of antibody and prolonging their persistence.

In addition, it is deemed advisable to study also the relationship of non-virion antigens to immunity. As a corollary, the relationship of such viral fractions and non-virion materials to the immunologic cross-reactions which occur between arboviruses should be investigated in depth, utilizing all of the recently developed immunologic methods.

Aside from the two major areas, viz. arboviruses and respiratory viruses, on which attention and effort should be centered, the joint panel feels that the very important problem of smallpox should be given consideration from at least two standpoints.

1. Research is highly desirable for the development of better laboratory methods for early and rapid diagnosis of smallpox and its differentiation from clinically similar diseases.
2. Research is also needed on methods that will serve to differentiate smallpox from vaccinia and also to differentiate it from other pox viruses.

After the October 1965 joint meeting in Honolulu, internal antagonism of Japanese virologists and members of the Science Council against the U.S.-Japan Cooperative Medical Science Program caused all Virology Panel members to resign, except the Chairman, Dr. Fukumi. Although another panel was not appointed until 1968, when the Japanese Virology Panel hosted a rabies conference in Tokyo, panel designees participated in a smallpox conference held in Honolulu in December 1967. After 1968, there is no official record of further internal antagonism among the Japanese virologists over participation in the U.S.-Japan Cooperative Medical Science Program.

Malnutrition Panel

When the Honolulu Conference was convened in October 1965, a United States Nutrition Group had been formed. Although a counterpart did not yet exist on the Japanese side, the U.S. members met with representatives of the Japanese Committee to discuss nutritional problems in Asia. The conclusions of that meeting report are recorded as follows:

Members of the Japanese committee in attendance agreed that a Japanese Panel on Malnutrition would be established. It was concluded that American and Japanese panels on malnutrition could then fruitfully confer on these important problems in the future. It was agreed that currently available public health measures to control malnutrition should be applied vigorously in the field. For example, enrichment of rice with thiamine and riboflavin will virtually eliminate beri-beri and ariboflavinosis among rice-eating peoples and should be universally adopted.

Legislation requiring this should be vigorously promoted. Although of less importance in Asia, white wheat flour should be enriched with B–complex vitamins. Iron should be added to the enrichment mixture for both rice and wheat since it will help to alleviate the widespread problem of iron deficiency anemia. The enrichment of salt with iodine is a cheap and practical method of proven effectiveness in eliminating endemic goiter and should be recommended. Even crude moist salt can be effectively iodized with potassium iodate and no special packaging or subsequent handling is required. Fluoridation of water supplies will also be helpful in reducing the high prevalence of dental disease.

Implementation of the research program outlined above should yield substantial gains in knowledge of some of the complicated problems of clinical nutrition, plant genetics, and food processing needed for the control of malnutrition in Asia.

The Joint Committee approved the addition of malnutrition to the program at the Hakone, Japan Meeting, August 17-19, 1966. Although the two panels did not have a unified set of goals and objectives, the U.S. Malnutrition Panel listed a number of research projects or interest areas that are listed below:

1. Genetic improvement of the nutritive value of the cereal grains rice, wheat, corn, sorghum, and millet by screening large numbers of commercial varieties and primitive parental types to identify lines with high content of the limiting essential amino acids in which the cereal is deficient.
2. Local development and testing of acceptable low-cost, protein-rich foods, utilizing ingredients potentially indigenous to the countries concerned.
3. Investigation of the distribution, importance, and methods of control of mycotoxin in foods consumed in countries of the area.
4. Studies of the elimination of natural toxic substances from legumes and other plant foods.
5. Determination of the effects of early malnutrition on the mental development, behavior, and learning of children in the area and the extent to which these are prevented by dietary supplementation.
6. Determination of the effect of malnutrition in children on resistance to infections and the mechanisms responsible.
7. Investigation of the prevalance, etiology, and effective prevention of nutritional anemias in children and adults of the region.

The Japanese and U.S. scientists shared a joint interest in the effect of malnutrition on infection and the study of nutritional anemias in children and adults (Nos. 6 and 7). In addition, the Japanese proposed the following:*

1. *Physiologic studies on protein deficiency*. Basic studies on protein deficiency as measured by urinary excretion of riboflavin will be followed by field work.
2. *Studies on amino acid metabolism in subclinical protein deficiency*. The free amino acid pattern, the ratio of essential to non-essential amino acids, and the concentration of amino acid metabolites in the blood and urine of humans will be investigated as biochemial procedures for the early diagnosis of subclinical protein deficiency. Further, the cystine content of hair, blood glutathione, and serum iron-binding capacity will be investigated. Both human and nonhuman experiments will be done.
3. *Biochemical studies on malnutrition*. The purpose of this project will be to elucidate the fundamental biochemical changes of malnutrition and the effect of treatment on these changes. Since the effect of metabolism of carbohydrates, lipids, and proteins are greatly influenced by the state of malnutrition, the metabolism of each nutrient and their interrelationships will be studied.

These represent the original research goals and objectives for the Panels on Cholera, Leprosy, Malnutrition, Parasitic Diseases, Tuberculosis, and Viral Diseases. The terminology of research goals and objectives evolved into *Panel Guidelines*.

Guideline Changes

Again it must be emphasized that guidelines are not static but are open to change to accommodate new research opportunities and technologies. They may also change as research priorities are shifted.

Through the years the original guidelines of most panels were greatly abbreviated, although the intent remained the same. The Joint Cholera Panels' guidelines appeared as the following:

The Cholera Panels considered all research on cholera and cholera-like diseases as relevant to the U.S.-Japan Cooperative Medical Science Program. The following were, however, the areas to which the panel assigned a higher priority:

1. *Methods of immunization*. Improvements of whole cell vaccines, development of effective adjuvants, isolation and purification of protective antigens, development of an effective toxoid.
2. *Pathogenesis and treatment*. Purification of responsible exotoxin, studies of mechanism of action of this toxin, and development of a pharmacologic means of rapidly blocking the action of the toxin; related investigations of basic mechanisms of secretion and absorption of water and electrolytes in the small intestine.
3. *Microbiologic investigations*. Studies of those properties of *V. cholerae* which are essential to its multiplication in the gut of the susceptible host, and of those properties of the host which render him vulnerable to *V. cholerae* infection.
4. *Enteric disease surveillance*. Development, in collaboration with the World Health Organization, of an enteric disease surveillance system throughout South and Southeast Asia.

In addition to research on cholera *per se*, the Cholera Panels felt that research in related areas of pathophysiology and immunology was essential to better understanding of pathophysiologic and immunologic mechanisms in cholera, and felt that investigations in the following areas were relevant to the U.S.-Japan Program: **gastrointestinal immunologic mechanisms** against gram negative enteric bacterial infections, and **mechanisms of electrolyte absorption** and excretion by the mammalian small intestine.

In October 1970, the Joint Cholera Panel modified its guidelines by deleting Item 4 and all that followed down to and including "mechanisms of electrolyte absorption...." Enteric disease surveillance, although important, was not really considered the responsibility of the Cholera Panels. Item 4 now contained a definition of cholera-like disease. This definition was added in order to emphasize the panels' recognition of the importance of stimulating research in other enterotoxin-mediated acute diarrheal diseases:

4. *Cholera-like diseases* are defined as acute diarrheal diseases in which the relationship between bacteria living in the lumen of the gut and a human host is such that the resulting interaction will not cause (1) tissue destruction, (2) symptoms of toxicity, or (3) bloody diarrhea. If the diarrheal disease becomes life-threatening, it is on the basis of fluid loss.

The panels felt that the revised guidelines, although fairly specific, still provided the program with a certain amount of flexibility which was considered essential.

---

*The above are summarized from a report written by Dr. N. Shimazono in "The Report of the Second Meeting of the U.S.-Japan Cooperative Medical Science Committee, 1966."

As research progressed, researchers did find organisms other than *V. cholerae* which caused cholera-like illness by producing a toxin/toxins such as enterotoxigenic *Escherichia coli*. Therefore, to clarify the guidelines, cholera-like diseases were described using *Escherichia coli (E. coli)* as an example. Recent studies showed that vibrios, including *V. cholerae*, have a distinct environmental life cycle which is probably an important determinant of epidemiology of vibrio infections. Improved understanding of the environmental ecology of vibrios, including those *V. cholerae* which cause epidemic cholera, may help elucidate practical mechanisms necessary to interrupt the transmission of disease. Therefore in 1977, the guidelines were revised to include other enterotoxigenic organisms and vibrio ecology. Item 4 became "Vibrio Ecology" and the defintion of cholera-like diseases was changed to a separate paragraph. The guidelines, which are current, are as follows:

CHOLERA

United States-Japan Cooperative Medical Science Program

All research on cholera as well as cholera-like diseases (e.g., enterotoxigenic *E. coli*) is relevant to the panel's interest. The following areas have been assigned a higher priority:

1. Methods of immunization. Improvements of whole cell vaccines, development of effective adjuvants, isolation and purification of protective antigens, and development of an effective toxoid;
2. Pathogenesis and treatment. Purification of responsible exotoxin, studies of mechanisms of action of this toxin, and development of a pharmacologic means of rapidly blocking the action of the toxin; related investigations of basic mechanisms of secretion and absorption of water and electrolytes in the small intestines;
3. Microbiologic investigations. Studies of those properties of *V. cholerae* which are essential to its multiplication in the gut of the susceptible host, and of those properties of the host which render him vulnerable to *V. cholerae* infection.
4. Vibrio ecology. Investigations of factors in the environment (e.g., food and water) outside the human host which influence population, distribution, growth rate and virulence of cholera vibrios.

Cholera-like diseases are defined as those in which the relationship between bacteria living in the lumen of the gut and a human host is such that the resulting interaction will not cause (1) tissue destruction, (2) symptoms of toxicity, or (3) bloody diarrhea. If the diarrheal disease becomes life-threatening, it is on the basis of fluid loss.

Leprosy

Prior to 1973, the Leprosy Panel was requested to abbreviate and sharpen the goals and objectives originally identified in 1965. The Joint Leprosy Panel's guidelines remained essentially unchanged, since the headings were the same, although the descriptive detail was omitted. In addition, no reference was made to the development of a formal fellowship program. The guidelines are as follows:

LEPROSY

United States-Japan Cooperative Medical Science Program

All areas of leprosy research are relevant to the program, although the following are of special interest:

1. Cultivation of growth of *Mycobacterium leprae* in tissue culture.
2. Animal transmission and experimental systems for growth of *M. leprae*;
3. Drugs against leprosy;
4. Chemoprophylaxis and measurement of minimal effective drug blood levels;
5. Vaccination;
6. Antigens similar to lepromin and the mechanism of lepromin reactions.

An attempt is made to recruit new scientists to work in the field of leprosy, and the program has enhanced and facilitated leprosy research by increasing the availability of leprosy biopsy material and immunologically deficient mice to qualified investigators.

7/83

The Leprosy Guidelines described in 1973 are still applicable in 1983, although the emphasis of program research shifts as scientific progress is made. The ability to propagate *M. leprae* in the armadillo, and the development of techniques to separate organisms from the tissue in pure form has dramatically influenced the direction of the research toward immunology and vaccine development.

Note: It is of interest to report that the Leprosy and Tuberculosis Panels originally held joint conferences in 1966, 1967, and again in 1968. However, it would appear that after 1968 a decision was made that these panels would convene separate conferences, since no mention is made of a joint conference after 1968. It was not until 1977 that another combined conference was planned and held in Boston. It was the occasion of the 12th Joint Meetings of the Leprosy and Tuberculosis Panels. One day was set aside for a combined meeting to discuss primarily the immunology of leprosy and tuberculosis. Expert immunologists not involved directly in leprosy and tuberculosis research were invited to participate. Thereafter, the panels continued to hold separate conferences until 1983, when the Joint Committee mandated that because these two panels shared research areas of mutual interest, they would henceforth hold combined meetings. Such Joint Conferences were held August 3-5, 1983, in Bethesda and August 27 through 31, 1984, in Tokyo.

Malnutrition

The guidelines (goals and objectives) described for the Malnutrition Panel were generally broad and lengthy. In 1973, as requested, the Malnutrition Panels identified the following areas for their collaborative research effort.

MALNUTRITION

United States-Japan Cooperative Medical Science Program

The malnutrition program has the following five research objectives:

1. Determination of the effects of malnutrition on physical and mental development, learning, behavior, physical capability, and performance.
2. Studies of the health significance and methods of prevention of nutritional anemias.
3. Influence of environment and host factors on requirements for health in Asian countries.
4. Investigation of the interactions of malnutrition and infection with special emphasis on the mechanisms responsible (U.S. panel only).
5. Studies on malnutrition due to metabolic disorders (Japanese panel only).

7/73

These guidelines represent the combined research interests of both sides. It is of interest to note that the special and different interests of the two panels are expressed in guidelines 4 and 5. The U.S. panel had a special interest in the effect of malnutrition on infection, and the Japanese panel was especially concerned with metabolic disorders.

The reduction of the incidence of malnutrition in Asia continues to be the major objective of the Joint Malnutrition Panels. However, since its inception in 1966, certain problems have been solved and other problems have been redefined, necessitating a series of changes in panel priorities. In 1978, a new priority was added dealing with health consequences of different (and changing) dietary patterns and food habits of developed countries and those which are undergoing rapid changes in their social, cultural, and physical environments. Selected studies are needed on the long-term consequences of changing dietary patterns, food habits, and the suggested relationship between dietary statistics and health disorders. The guidelines now in effect are:

MALNUTRITION

United States-Japan Cooperative Medical Science Program

The malnutrition program has the following five research objectives:

1. Influence of environmental and host factors on requirement for health in Asian countries.
2. Studies of the health significance of iron deficiency and methods for its eradication.
3. Investigation of the interactions of nutrition and infection with special emphasis on the mechanisms responsible.
4. Determination of the effects of nutrition on physical and mental development, behavior, physical capability, and work performance.
5. Health consequences of different (and changing) dietary patterns and food habits.

These guidelines are under active revision by the joint panel at the time of publication of this report.

7/78

Parasitic Diseases

The Parasitic Diseases Panels limited their research efforts to only filariasis and schistosomiasis. In 1973, the guidelines were abbreviated and organized as follows:

PARASITIC DISEASES

United States-Japan Cooperative Medical Science Program

The scientific interests of the Parasitic Diseases Panel are specifically confined to two diseases: filariasis and schistosomiasis. Animals infected with these two parasites are made available to encourage and awaken scientific interest in these two disease entities.

Filariasis

1. Mechanisms of the pathogenesis of any filarial infection:
   - direct damage due to tissue invasion and toxicity of metabolic products of worms.
   - hypersensitivity mechanisms;
   - concomitant microbial infections.

Most of the foregoing research involves selected animal model systems using filarid species such as *Dipetalonema* and *Dirofilaria*.

2. Biochemical and physiological studies on a variety of filarid parasites (Filarioidea), including both adult parasites and larvae developing in intermediate hosts, as well as worm stages maintained *in vitro*.
3. Immunological and serological research on human filarids or related animal species.
4. Chemotherapy and pharmacology on related animal filarids and clinical studies and clinical trials of drugs in human filariasis.

To be included in the foregoing investigations are a variety of host-filaria studies regardless of species or locale, including vector-parasite relations, vector physiology and genetics, and other basic studies. However, excluded from this research purview are local epidemiological or ecological investigations of vectors unless they are proposed to be done in Asia.

Schistosomiasis

Studies of the three species of human schistosomes, *S. mansoni*, *S. haematobium*, and *S. japonicum*, are undertaken on the same basis, and with the same exclusions, as filariasis above.

7/73

The panels were satisfied that these guidelines were broad enough for each disease to provide research flexibility.

In 1975, the Parasitic Diseases Panel revised its guidelines in order to emphasize and to clarify their research areas of interest. These changes were approved by the Joint Committee in July 1976. The guidelines are as follows:

PARASITIC DISEASES

United States-Japan Cooperative Medical Science Program

The Parasitic Diseases Panel has primary interest in research on all forms of schistosomiasis and filariasis. Sponsored studies share a long-term goal of alleviation of these two diseases. Strategies include vector control, interruption of the parasite life cycle, and reduction of the amount and effects of infection in man by vaccination, chemoprophylaxis, or other modalities. Both basic and applied work is supported in the following areas:

> VECTOR BIOLOGY, e.g., genetics, reproduction, metabolism, behavior; PARASITE BIOLOGY of all stages of schistosome and filarial worms; VECTOR-PARASITE and MAMMAL-PARASITE INTERACTIONS; PATHOGENESIS in mammalian hosts, both human and non-human; IMMUNOLOGY with emphasis on stimulation of resistance; and PARASITE BIOCHEMISTRY and CHEMOTHERAPY and related subjects.

In addition, the panel is concerned with SUPPLY OF PARASITES AND INFECTED ANIMALS to investigators and with AVAILABILITY OF RESEARCH WORKERS in schistosomiasis and filariasis.

REGIONAL FIELD STUDIES on epidemiology or ecology of schistosomiasis and filariasis must be relevant to the interests of the sponsoring panels.

Revised 1976

These guidelines were once again revised in 1984 as a result of a symposium held at the National Institutes of Health in Bethesda, Maryland in July 1983, to review the state-of-the-art of parasitic diseases research.

Consequently, the guidelines were revised so as not to limit panel research to only schistosomiasis and filariasis but expanded to include malaria and other important protozoal and helminthic diseases: The revised guidelines are:

PARASITIC DISEASES

United States-Japan Cooperative Medical Science Program

The Parasitic Diseases Panels have a primary interest in research on selected helminthic and protozoal diseases with special emphasis on vector-borne parasitic diseases such as but not limited to malaria, schistosomiasis, and filariasis. Sponsored studies share a long-term goal of alleviation of these diseases. Strategies include vector control, interruption of the parasite life cycle, and reduction of the amount and effects of infection in man by vaccination, chemoprophylaxis, or other modalities. Both basic and applied work is supported in the following areas:

EPIDEMIOLOGY;
VECTOR BIOLOGY AND CONTROL;
PARASITE BIOLOGY;
HOST-PARASITE INTERACTIONS;
PATHOLOGY;
IMMUNOLOGY;
BIOCHEMISTRY; AND
CHEMOTHERAPY.

Special emphasis will be given to studies designed to explore the role of membrane chemistry and antigen differentiation in infection and immunity. In addition, the panel is concerned with SUPPLY OF PARASITES AND INFECTED ANIMALS to investigators and with AVAILABILITY OF RESEARCH WORKERS in these fields.

2/10/84

Tuberculosis

The original goals and objectives (1965) of the Tuberculosis Panels were not described in detail, although broad, general concepts were cited. By 1973, the guidelines were nicely defined.

TUBERCULOSIS

United States-Japan Cooperative Medical Science Program

Tuberculosis research is concerned primarily with projects relating to the pathogenesis and immunological aspects of tuberculosis. Subjects of special interest are immunity and hypersensitivity in tuberculosis, the analysis of cell substances of tubercle bacilli which might be related to immunity, and experimental models for the study of tuberculoimmunity:

1. Fractionation and identification of biologically active constituents of tubercle bacilli;
2. Pathogenesis of tuberculosis
   — experimentally induced tuberculosis
   — host-parasite relationship
   — atypical mycobacteria;
3. Immunity and hypersensitivity in tuberculosis, including mechanisms and
   — immunochemical manipulation of mycobacterial antigens, haptens, and serology
   — immunoglobulin responses of experimental animals to infection and immunization
   — tuberculin hypersensitivity responses
   — testing of new potential agents for immunization
   — cytopathology of immunization
   — vaccination against tuberculosis
   — mechanisms of acquired cellular resistance;
4. Non-specific immunity and hypersensitivity stimulated by mycobacteria.

To further these studies, special reagents are made available for research purposes only to qualified investigators: living BCG bacterial cells, lyophilized PPD tuberculin, characterized mycobacterial cultures of research interest, a culture filtrate reference antigen, and polyvalent reference antiserum by which investigators are able to standardize laboratory produced material.

In 1978, the Tuberculosis Panels revised their guidelines to emphasize their special interest in the pathogenesis and immunological aspects of the disease. The research projects and grants funded by both sides involved new and sophisticated immunological approaches, techniques, and methodologies. The revised guidelines approved by the Joint Committee in July 1979 are as follows:

TUBERCULOSIS

United States-Japan Cooperative Medical Science Program

Tuberculosis research is concerned primarily with projects relating to the pathogenesis and immunological aspects of the disease. Subjects of special interest are immunity and hypersensitivity, the analysis of cell substances of tubercle bacilli which might be related to immunity, and experimental models for the study of tuberculoimmunity:

1. *Biology of mycobacteria*
   a. Fractionation and identification of biologically and immunologically active constituents of tubercle bacilli
   b. Genetics and physiology
   c. Mechanisms of drug action and development of resistance

2. *Pathogenesis of tuberculosis and other mycobacterioses*
    a. In man and experimental models
    b. Host-parasite relationship
    c. Environmental and nutritional factors

3. *Immunology of tuberculosis*
    a. Mechanisms of hypersensitivity, anergy, and acquired resistance
    b. Immunoglobulin response to infection and immunization
    c. Activation of nonspecific effector mechanisms by mycobacterial and other products
    d. Adjuvant properties of mycobacterial and other products

4. *Immunoprophylaxis*
    a. Live vaccines
    b. Killed vaccines
    c. Natural and synthesized mycobacterial fractions

To facilitate these studies, special reagents are made available only for research purposes to qualified investigators: lyophilized PPD tuberculin, characterized mycobacterial cultures of research interest, a culture filtrate reference antigen preparation and polyvalent reference antiserum with which investigators are able to standardize laboratory produced material.

7/25/79

Viral Diseases

The goals and objectives originally (1965) identified for the Viral Diseases Panels were extremely broad and included six arboviruses, although it was decided to concentrate on Japanese encephalitis virus and the dengue complex, plus the following respiratory viruses: influenza, parainfluenza, respiratory syncytial, and adenoviruses. Interest was also expressed in performing research on smallpox and eventually on rabies. Workshops and conferences were held on smallpox, myxovirus infections, viral vaccines with special emphasis on Japanese encephalitis, rabies, and the pathogenesis of arbovirus infections with emphasis on the cause of dengue hemorrhagic fever. Additional conferences included a potpourri of viruses.

It became evident to the Joint Committee that the Viral Diseases Panels' research interests and programs were much too diffuse to be effective. This information was transmitted to the Panel Chairmen. In 1973, the guidelines were modified as follows:

VIRAL DISEASES

United States-Japan Cooperative Medical Science Program

Rabies

Basic, mission-oriented, or applied studies including control measures, diagnostic techniques, virus reservoirs, and prophylactic procedures.

Dengue

Studies of the epidemiology and pathogenesis of hemorrhagic fever, development of vaccines using selected viral structural components as the primary antigen, basic immunology with respect to classification, biophysical and biochemical studies.

Arboviruses

Studies on the structural elements of arboviruses, including isolation and identification of antigenic components.

Also, a joint panel review was initiated by the Subcommittee on Program Review and Planning in 1973 and completed in 1974. As a result of this review, a recommendation was made by the Joint Subcommittee and Committee that the two panels develop a truly collaborative program during the next year, and the reviewers were requested to monitor this activity and to report their findings to the Joint Committee in 1975. These panels accepted this charge and presented a unified effort in 1975, and this cooperative effort has continued to intensify over the years.

In 1978, the guidelines of the Viral Diseases Panels were expanded to include viral gastroenteritis, since this important health area was excluded from the Cholera Program. The composition of the panels was changed to accommodate this new area, and the research program that evolved has been very successful. The present guidelines read as follows:

VIRAL DISEASES

United States-Japan Cooperative Medical Science Program

Rabies

Basic, mission-oriented, or applied studies including control measures, early and late diagnostic techniques, virus reservoirs, and prophylactic procedures.

Dengue

Studies of the epidemiology, early confirmatory diagnosis, and pathogenesis of hemorrhagic fever. Development of vaccines using selected viral structural components as the primary antigen; basic immunology with respect to classification; biophysical and biochemical studies.

Arboviruses

Studies on the structural elements of arboviruses, including isolation and identification of antigenic components.

Viral Gastroenteritis

Epidemiologic investigations of infant and adult gastroenteritis using virological techniques; diagnostic methods, antigenic characterization (including human and related animal viruses), and studies on host immune response requisite for vaccine development, epidemiologic surveillance, or control; detection, propagation, assay, and characterization of putative agents for man; and studies of pathogenetic mechanisms and predisposing factors that could lead to improved prophylaxis, therapy, or control.

7/78

Methods for Evaluating Environmental Mutagenesis and Carcinogenesis

In 1972, a seventh panel on Methods for Evaluating Mutagenesis and Carcinogenesis was added to the U.S.-Japan Program. This panel is usually referred to as the Environmental Mutagenesis and Carcinogenesis Panel or shortened even further to the Environmental Panel. The approved guidelines are as follows:

METHODS FOR EVALUATING ENVIRONMENTAL MUTAGENESIS AND CARCINOGENESIS

United States-Japan Cooperative Medical Science Program

There is expanding evidence that human health will be increasingly influenced by chemical pollutants introduced by man into his environment. The two areas of environmental disease selected by the program are mutagenic and carcinogenic effects.

Detection of chemical pollutants with mutagenic and carcinogenic activity:

— establishing a registry of known chemical mutagens;
— development of test systems to screen chemicals for mutagenic activity;
— support of studies to determine how to extrapolate data from experimental mutagenicity test to man; and
— development of test systems to determine the correlation between carcinogenic and mutagenic activity.

Surveillance to determine whether pollutants have caused an increase in the genetic load in man:

— development of methodology for monitoring the human population to determine whether there is any change in the genetic load; and
— development of methodology for exploratory and intensive epidemiological investigations in human populations suspected or known to have been exposed to a mutagenic or carcinogenic agent.

The early work of the Environmental Panel was directed toward developing satisfactory methodology for the detection of mutagenic and carcinogenic compounds in the environment. For example, AF-2, a food preservative used in Japan, was banned because it was found to be a potent mutagen and potentially dangerous to human health. With the development of effective test systems, these panels revised their guidelines to concentrate on what effect mutagens and carcinogens had on **industrialized** and **selected populations** in **Asia**. The revised guidelines were approved by the Joint Committee in July 1979, and they are as follows:

Environmental Mutagenesis and Carcinogenesis United States-Japan Cooperative Medical Science Program

There is expanding evidence that human health will be increasingly influenced by chemical pollutants introduced by man into his environment. The two areas of environmental disease selected by the program are mutagenic and carcinogenic effects.

A. Detection of chemical pollutants with mutagenic and carcinogenic activity:

1. development, refinement, and validation of mutagenicity assay systems for use in mass screening programs;
2. support investigations which improve our ability to make human risk estimations. Areas of work include: comparative mutagenesis and extrapolation to man, somatic and germ cell dosimetry, pharmacokinetics, and relative impact of various genetic lesions;
3. support investigation of factors which modify mutagenic activity;
4. support investigations of structure-activity relationships of both mutagens and carcinogens.

B. Surveillance to determine the occurrence and effects of mutagens and carcinogens in the environment, **with emphasis not only on industrialized populations but also on unusual populations in Asia.**

1. development of methodology for monitoring the human populations for mutagens;
2. application of human monitoring methods to determine baseline mutation frequencies and to monitor high-risk subpopulations such as chemotherapy patients, workers exposed to known or suspected mutagens, and individuals genetically predisposed to genetic damage and carcinogenesis;
3. development of methodologies for monitoring the work place and general environment (air, water, and soil) for the presence of mutagenic or carcinogenic pollutants;
4. development of methodology for epidemiological investigations of human populations suspected or known to have been exposed to mutagenic or carcinogenic agents.

7/25/79

The guidelines were reviewed by the panels in light of past research accomplishments and future plans. As a result, the guidelines were revised and submitted to the Subcommittee on Program Review and Planning at their February 1985 meeting for review and comment. The Subcommittee approved and will recommend to the Joint Committee in July that the following guidelines be adopted:

Environmental Mutagenesis and Carcinogenesis United States-Japan Cooperative Medical Science Program

There is expanding evidence that chronic human diseases, including cancer, can be caused by man-made and natural chemicals as well as other agents in the human environment. This program focuses on two aspects of such environmental disease: mutagenicity and carcinogenicity. Approaches to these can be broadly divided into laboratory and epidemiologic studies. For both, one goal is to apply and transfer knowledge to world populations, particularly in Asia, developing countries, and high-risk areas.

A. Laboratory approaches to the detection, prediction, interpretation and mechanisms of the mutagenic an carcinogenic properties of chemical and physical agents.

1. Development, refinement, validation and application of laboratory assay systems for assessing mutagenicity and carcinogenicity.
2. Systemic collection and evaluation of data-base on mutagens and carcinogens.
3. Studies of the relationships:
    a. between mutagenicity and carcinogenicity
    b. in the toxic responses of various species, especially microbial, rodent, and human
    c. between somatic and germ cell mutagenicity
    d. among types of genetic lesions, e.g. gene mutations and chromosomal aberrations
    e. between initiation and promotion in carcinogenesis
    f. between chemical structure and toxicity
    g. between pharmacokinetics/metabolism and toxicity
4. Investigation of factors which modify mutagenic and carcinogenic activity, including dose-time effects, repair processes and multi-agent interactions.

B. Epidemiologic approaches to understanding and preventing environmental mutagenesis and carcinogenesis in human populations.

1. Development, refinement, and validation of methods to determine exposure, DNA dosimetry, and somatic and germ cell mutations' early stages of carcinogenesis in human populations.
2. Application of human monitoring methods to determine baseline frequencies of mutation or other monitored endpoints and to monitor high-risk groups such as chemotherapy patients, workers exposed to known or suspected mutagens and carcinogens, and individuals or families genetically predisposed to genetic damage and cancer.
3. Promote interdisciplinary studies of human mutagens and carcinogens.
4. Development of qualitative and quantitative methods for monitoring the work place and the general environment (air, water, soil, food) for the presence and levels of mutagenic or carcinogenic agents.
5. Furthermore, epidemiologic methodology and expansion of epidemiologic resources to measure mutagenicity and carcinogenicity by agents in the human environment.
6. Combining of laboratory studies of agents, human hazards, and human exposure into a sound, scientifically based risk analysis for mutagenicity and carcinogenicity.

2/85

Hepatitis

The Joint Committee approved the addition of viral hepatitis to the Program in July 1979. This was the second program area to be added since the inception of the U.S.-Japan Cooperative Medical Science Program in 1965. The guidelines for hepatitis research are as follows:

HEPATITIS

United States-Japan Cooperative Medical Science Program

Viral hepatitis is a serious health problem in many areas of the world including Japan, the United States, and Asia. Two known types most commonly recognized are types A and B. A third type of viral hepatitis has been identified and by exclusion is referred to as non-A non-B hepatitis. The long-term objective of the program is to define conditions for the control of viral hepatitis by improved environmental conditions, therapy, or prophylaxis. Research areas of high interest to the program are:

1. Biochemical and biophysical characterization of agents including those of non-A non-B hepatitis.
2. Seroepidemic studies in Japan, the United States, and Asian countries to identify prevalence and incidence of infections caused by the various hepatitis viruses. Implicit in these objectives is appropriate work on *in vitro* cultivation of hepatitis agents.
3. Development and evaluation of vaccines and anti-viral compounds for prevention and treatment of disease with particular emphasis on therapeutic measures for chronic carriers of type B virus.
4. Long-term studies to determine the incidence of hepatoma as a sequela of chronic viral hepatitis infection

7/25/79

At a March 1984 meeting, the Joint Hepatitis Panel expanded its guidelines slightly to include the hepatitis delta virus and to add cirrhosis as a sequela to chronic viral hepatitis. The modified guidelines are:

HEPATITIS

United States-Japan Cooperative Medical Science Program

Viral hepatitis is a serious health problem in many areas of the world including Japan, the United States, and Asia. Two known types most commonly recognized are types A and B. Additional types of viral hepatitis have been identified and by exclusion are referred to as non-A non-B hepatitis. Another recently identified agent is the hepatitis delta virus. The long-term objective of the program is to define conditions for the control of viral hepatitis by improved environmental conditions, therapy, or prophylaxis. Research areas of high interest to the program are:

1. Biochemical and biophysical characterization of agents.
2. Seroepidemic studies in Japan, the United States, and Asian countries to identify prevalence and incidence of infections caused by the various hepatitis viruses. Implicit in these objectives is appropriate work on *in vitro* cultivation of hepatitis agents.
3. Development and evaluation of vaccines and anti-viral compounds for prevention and treatment of disease with particular emphasis on therapeutic measures for chronic carriers of type B virus.
4. Long-term studies to determine the incidence of cirrhosis and hepatocellular carcinoma as a sequela of chronic viral hepatitis.

3/28/84

Immunology

The last area to be added to the U.S.-Japan Cooperative Medical Science Program was immunology. Since this was discipline oriented rather than disease oriented, it was given the name of Immunology Board to reflect this difference. The Immunology Board and its guidelines were approved by the Joint Committee in July 1981. The addition of immunology, the third since 1965, brings the total number of research areas involved in the U.S.-Japan Program to nine. The guidelines are as follows:

IMMUNOLOGY

U.S.-Japan Cooperative Medical Science Program

Immunology has made rapid advances in the last decade. The recent introduction of the techniques of somatic cell hybridization, molecular genetics, and long-term culture of immunocompetent cells to the study of the immune system has caused an unprecedented increase in our understanding of this field. Such progress in fundamental immunology has the potential to revolutionize the diagnosis, treatment, and prevention of immunologic and infectious diseases, including those being studied under the auspices of the U.S.-Japan Cooperative Medical Science Program.

A long-term objective of the Immunology Board is to study and elucidate the cellular and molecular mechanisms of the immune response and to define effective approaches for the manipulation of the immune system.

In order to achieve these purposes, the Board will focus its concern on the following research areas:

1. Molecular studies of the genetic organization of the lymphoid system.
2. Cellular and molecular studies of lymphocyte activation, proliferation, and differentiation.
3. Immunogenetic approaches to the study of lymphocyte interactions, with emphasis on the role of histocompatibility gene products in the regulation of the immune response.
4. Cellular and molecular studies of the regulation of the immune response, with emphasis on the role of histocompatibility gene products in the regulation of the immune response.
5. Application of techniques for the development of stable cell lines and of somatic cell hybrids to the study of the regulation of lymphocyte activation and function and to the preparation of monoclonal antibodies, lymphocyte receptors and specific, as well as non-specific lymphocyte products.
6. Mechanisms to support the interaction of immunologists and the exchange of information and material between the two countries.

7/20/81

CRITERIA FOR PANEL REVIEW

The plan for Program reviews specifies that the Co-Chairmen of the Joint Committee appoint a subcommittee comprised of members of each delegation to review and to evaluate the accomplishments of existing panels and board with respect to the overall goals of the U.S.-Japan CMSP. Primary attention is directed toward research guidelines which have been identified by the panels/board and approved by the Joint Committee. Criteria regarded as essential for a proper evaluation of the respective guidelines as they relate to the current research programs are:

1. Does the research outlined in the guidelines remain relevant to the objectives of the U.S.-Japan CMSP?

2. How well does the scientific program fulfill its research guidelines? Is the research program sufficiently concise to ensure achievement of its designated basic and practical objectives, and does it avoid diffuseness which could lead to ineffectiveness?

3. How appropriate are these guidelines in the light of new and current knowledge, needs, and opportunities?

4. Realistically in terms of scientific applications, can these objectives be achieved by pursuing further the approaches currently being utilized?

5. Is there a reasonably identifiable timeframe wherein the research objectives of the guidelines can or should be reached, modified, or terminated?

It is understood that the respective U.S. and Japanese reviewers will prepare a combined report carefully evaluating the progress of the panel or board under review in accordance with the five review criteria. It is the prerogative of the reviewers to invite outside consultants to assist in the evaluation process if deemed advisable. The review report is submitted initially to the Subcommittee on Program Review and Planning for comment and approval before being presented to the Joint Committee for a final decision. This decision is based on the following:

When compared with other potential program areas which also may offer promise for progress and accomplishment, should the panels/board under review be:

a. modified
b. combined with another program area
c. reduced or expanded in scope
d. recommended for other sponsorship
e. phased-out in an orderly manner
f. continued as currently programmed?

Each panel/board review is arranged in accordance with the following format: introduction, list of present and past members, summary of research progress under each guideline, and a concise evaluation of whether the research is responsive to the five criteria previously cited.

In this report only a portion of the review for each panel is presented.

PANEL REVIEWS

Leprosy Panel

Panel Reviewers: Dr. Tadao Shimao
Dr. Robert E. Shope

1. The panel's research remains relevant to the Program, because leprosy afflicts an estimated 9 million people in the tropical and subtropical regions of the world. About one-fourth of these are crippled, and new cases are recognized annually. In addition, evidence indicates that *M. leprae* is developing resistance to dapsone, the drug most commonly used for treatment. *In vitro* cultivation of the agent has not been accomplished and no vaccine is available. Future research on leprosy will clearly benefit both the United States and Japan, as well as the developing nations of Asia.

2. The guidelines adopted by the joint panels has in general been closely followed. The emphasis of research has properly been directed to those aims such as animal systems for growth of *M. leprae* and immunology, where available technical advances have permitted the most rapid progress. The panel is to be commended for persisting in trials to cultivate *M. leprae in vitro* in spite of the elusiveness of this goal. Greater emphasis is needed in the future on the development of chemoprophylaxis and, especially, aspects relating to the development of drug resistant *M. leprae*.

3. The field of leprosy is ideally suited to take advantage of new advances in immunology, microbiology, and biochemistry. It is entirely possible that by the time of the next review, immunotherapy may be a practical outcome of leprosy research, and antigens may be identified by biochemical and monoclonal antibody techniques to permit a successful antigenically defined vaccine. If protective antigens can be defined, DNA recombinant technology is available to implement large-scale production. The objectives remain completely appropriate to take advantage of these new technologic advances.

4. It is evident that the panel has made a concerted effort to attract young investigators with competence in new technology to the field. This trend is applauded, and if it can be continued, the approaches to the stated objectives will con-

tinue to be modern and relevant. An excellent example is the application of technology in *Neisseria* research to *M. leprae* antigen purification and characterization.

5. Barring a major event such as the development and mass production of an antigenically defined vaccine, or an inexpensive, safe, bacteriocidal drug, it is not anticipated that the panel's objectives will be satisfied within the next 5 years. The Special Programme for Tropical Diseases of the World Health Organization has targeted leprosy for research emphasis. The added resources to the field are welcome. The U.S.-Japan Panels clearly have an essential mission because they have broader aims and a wider framework of research than the THELEP and IMMLEP Panels of WHO, and offer a forum which the WHO Programme cannot match.

The Joint Committee recommended that the Joint Leprosy Panel be continued.

Parasitic Diseases Panel

Panel Reviewers: Dr. Barry R. Bloom
Dr. Manabu Sasa

1. While schistosomiasis and filariasis do not represent major health problems in either the United States or Japan, the panel's research remains relevant because the diseases affect hundreds of millions of people in developing nations of Asia. In addition, building on the base of classical life-cycle expertise, the possibility of newer immunologic and molecular approaches for the study of these diseases is attracting many younger investigators into the field in both countries, and the panel serves as a focus for intellectual interactions. While particularly effective new chemotherapeutic agents against some forms of schistosomiasis have been recently developed, more cost-effective drugs and development of vaccines for prevention of both schistosomiasis and filariasis are greatly needed.

2. The guidelines adopted by the joint panel have been closely followed. The emphasis of research was initially directed toward establishing appropriate animal and vector models for study, and supply of vital materials to interested investigators. In recent years, a transition has been made to introducing newer concepts and methodologies, especially in immunology, and this is to be commended and encouraged. In particular, greater emphasis should be placed on use of monoclonal antibodies for delineation of protective and species specific antigens for both parasites, that would have relevance both for seroepidemiology and vaccine development. It is also to be hoped that with such reagents, application of molecular biology and recombinant DNA techniques could accelerate development of diagnostic reagents and possibly protective vaccines, or at least that possibility should be explored. Finally, progress in the immunoregulatory changes in both diseases should provide new insights into pathogenesis and possibilities for intervention to diminish pathological changes. Vector control remains clearly important for both diseases for the foreseeable future.

3. Helminithic infections are extremely complex diseases, both in terms of life cycle requirements, pathogenesis, and protection. New advances in immunology, microbial chemistry, and chemotherapy, as well as molecular biology, will undoubtedly have enormous impact in accelerating knowledge of these diseases and in revealing new methods of intervention and prevention. In schistosomiasis, new drugs have been introduced and are in field trials which have potential therapeutic value with minimum toxicity, and the possibility of a vaccine, which could have real epidemiological impact, must be supported if protective antigens and immunization regimens can be defined in experimental systems. In filariasis, much less is understood both in terms of pathogenesis, adaptation of parasites to the vector and hosts, and protective mechanisms, and these must be explored at the basic research level. The search for new effective drugs on filariasis should be given high priority.

Because of fiscal restraints for research in tropical diseases in the United States, it has generally been agreed that the focus of the Parasitology Panel's activities on only two diseases has provided an important concentration of intellectual and scientific activity on filariasis and schistosomiasis, and prevented consideration of helminthic diseases from being diluted by the wide range of parasitic diseases. On the other hand, because of the importance of the Japanese panel's activity for recognition of tropical diseases research, and the recent progress in newer approaches to the immunology and molecular biology of malaria, it is seen as advantageous to the pursuits of the Japanese panel to enlarge its scope of activities to include research in malaria under the panel's guidelines. Thorough consideration of the implications of these choices of objectives and scope should be taken into consideration.

4. The panel has made significant contributions, both in terms of the research programs encompassed, the contracted supply of crucial reagents, and, in earlier years, workshops and minisymposia focused on key understanding problems in the field. Greater understanding in each of these areas of the stated objectives will continue, both under the aegis of the U.S.-Japan Program, and from other sources. It is to be hoped that the panel will attract a broader range of investigators working in these areas to its meetings, particularly those with competence in the newer technology. One example would be investigators working on monoclonal antibodies to define stage specific and species specific as well as protective antigens, and molecular biologists interested in using recombinant DNA techniques that might be helpful in the development of vaccines, or antigens for widescale epidemiological surveys. For these purposes, it is crucial that workshops and symposia be expanded, as well as the numbers of scientists who can attend.

5. While it is always possible that inexpensive, safe, schistosomocidal or filaricidal drugs will be developed, and such drugs would have therapeutic potential, they are unlikely to change the epidemiologic picture. Obviously, study of vector biology and development of vaccines will still be required in both schistosomiasis and filariasis for the foreseeable future.

While the U.S.-Japan panel once had a unique function, the magnitude and importance of schistosomiasis and filariasis in the developing world has been recognized by a number of international and national organizations which sponsor research in these areas. The Special Program for Research and Training in Tropical Diseases of WHO, the Great Neglected Diseases of Mankind Program of the Rockefeller Foundation, and the support of the Edna McConnell Clark and other foundations, provide additional support for research in these two diseases. In addition, the summer course in the Biology of Parasitism offered at the Woods Hole Marine Biological Laboratory has stimulated bright young investigators often trained in molecular biology and immunology to enter the field. Last year, the first Gordon Conference on parasitism was held; it had the greatest number of applicants of any previous summer conference, and admission had to be restricted to only 20% of qualified applicants. While the panel no longer controls the major funding for research in these two diseases, or even serves as the sole focus of scientific interactions in these diseases, it does represent the major focus of U.S.-Japan collaboration in these diseases, and has led to exchange visits and training in important techniques to a considerable number of scientists who have carried out important research in both countries.

In response to 15 letters sent to investigators in the fields of schistosomiasis and filariasis research, as well as to distinguished parasitologists working on other diseases in the United States, the replies have indicated mixed feelings about the importance of the panel's functions. Some investigators believed that panel activities have a lesser impact on research in those fields than other forums, and that most of the communications presented at the U.S. panel meetings are now being presented simultaneously in other scientific meetings as well. Other respondents believed strongly that in terms of the initiation of key contracts, workshops, and exchange of scientists, as well as communication with their Japanese colleagues, panel activities are unique and vital as the underpinning for all research in these diseases at the present time.

If appropriate support is given for the panel's activities, including support of contracted research, grant-related research in areas, involving newer scientific approaches to the problems of these diseases, and support for workshops and symposia on topics of fundamental interest and importance, there is little question that the panel can maintain its place as an important focus on schistosomiasis and filariasis, and will continue to serve a vital and important function, particularly to young investigators in those countries. In addition, it is important to augment its activities by developing new opportunities for international cooperation in tropical diseases research, both through the existing funding agencies, and directly in disease endemic countries.

The Joint Committee recommended that the panel be continued but that its guidelines be revised to take advantage of new research opportunities. To this end, it was recommended that the joint panel convene a symposium in 1983 to review the current status of parasitic disease research.

1982

Viral Diseases Panel

Panel Reviewers: Dr. Hideo Fukumi
Dr. Robert E. Shope

1. Rabies, dengue and other arboviruses, and rotaviruses cause diseases which are not only continuing problems, but also in some instances such as dengue and rabies are increasing problems. An outside review done in 1976 encouraged the Program to examine diseases common to the United States and Japan. The research on viral gastroenteritis embodies this concept and has served exceptionally well to maintain relevance of the research to program aims. Rabies, dengue, and other arboviral diseases, especially Japanese encephalitis, are increasingly severe problems in Asia. In addition, dengue hemorrhagic fever in 1981 became a New World problem and threatens the United States itself.

2. The panel's scientific program fulfilled very closely the objectives as set out in the guidelines. The progress in viral gastroenteritis is a result of careful planning and joint Japanese and U.S. cooperation to achieve a common goal. It sets a challenge as a model for other joint efforts in the future.

The Program is targeted and overall the resources available are sufficient to ensure progress in the three major areas: rabies, dengue and other arboviruses, and viral gastroenteritis.

In the area of other arboviruses, the guidelines emphasize "studies on the structural elements of arboviruses, including isolation and identification of antigenic components." In practice, a wide variety of other research, not directly related to the guidelines, was undertaken. These projects have resulted in useful knowledge and have certainly been effective; could they be more effective if they were less diffuse?

3. The guidelines on rabies, dengue, and viral gastroenteritis remain extremely appropriate. For rabies, a need continues for basic mission-oriented control measures, early diagnosis, and prophylaxis. The major need for control in Asia is an inexpensive vaccine for dogs. This has only partially been achieved. While diagnosis has been refined, the need, given new technology, is for developing rapid and sensitive techniques. Post-exposure prophylaxis also can be improved by applying new methods for lowering cost and increasing efficacy. Consideration should be given for initiation of joint studies to compare the epidemiology in fauna which support the epizootics of rabies in the United States and Asia. At present, very little is known about either the dynamics of the animal populations or about the epizootic transmission of rabies virus.

For viral gastroenteritis, progress on the stated objectives with rotaviruses has been gratifyingly rapid. The opportunities are not yet exhausted, however. In fact only the surface has been scratched in the study of other diarrheal disease viruses such as Norwalk agent and calciviruses.

The joint panel should consider modifying the aims of the "other arboviruses" guidelines to include study of the existing or potential flavivirus problems which the two countries face. In Japan this is Japanese encephalitis virus, which is still endemic; in the United States this is St. Louis encephalitis. The basic epidemiology and especially the mechanism of persistence of these viruses remains an enigma. Also, epidemics of Japanese encephalitis have become more common and more extensive in the last 5 years in Asia. In some areas they are of alarming proportions. This situation calls for a new look at the development of an inexpensive vaccine, possibly live attenuated or genetically engineered. Japanese encephalitis and St. Louis encephalitis are closely related in their epidemiology and replication. This should be a fruitful area for cooperative study by the joint panels.

4. New technology, especially monoclonal antibodies and the new ability to make sub-unit vaccines by genetic manipulation or by polypeptide synthesis can now be applied to each of the major guideline areas. In particular these should be considered in a new program to study other flaviviruses such as Japanese encephalitis and St. Louis encephalitis. Molecular epidemiology has also now become possible for rabies, dengue, other flaviviruses, and rotaviruses. Application to these stated objectives is urgently needed and is feasible.

5. It is unlikely that each of the objectives will be reached within the next 5 years, although frequent review is needed.

Conceivably a vaccine or vaccines for the four rotavirus serotypes will be developed in this time frame, but even here, other viral gastroenteritis agents will need to be studied.

Recommendation for continuation of the Viral Diseases Panel was agreed upon by the Joint Committee.

1983

Tuberculosis Panel

Panel Reviewers: Dr. Theodore E. Woodward
Dr. Shiro Someya

1. In 1965, the signatory countries "recognized their mutual concern for the health and well-being of all the peoples of Asia" and agreed to undertake a greatly expanded, joint cooperative research effort in the medical sciences, which was dedicated to concentrating on health problems in Asia.

The World Health Organization stresses that tuberculosis remains a major problem throughout the world. Its prevalence is estimated to be 15-20 million active cases, with about 10 million persons developing tuberculosis annually. Annual deaths number some 3 million. In the Asian subcontinent recent reports estimated 8 million active cases of tuberculosis, of which 2 million are sputum positive; a large number among the apparently healthy population are expected to develop active illness each year. The failure of the South Indian BCG trial raised grave concern about the efficacy of BCG vaccination. This realization was compounded further by the advancement of numerous differing hypotheses, the testing of which **might** reveal the valid cause of the vaccine failure. This is a reflection of the paucity of current reliable information.

The magnitude of the problem of tuberculosis and its alleviation, delineated in 1965 when the U.S.-Japan Program was inaugurated, has not diminished. A wide spectrum of research programs to which the United States and Japanese Tuberculosis Panel is committed are appropriate for further pursuit of the established objectives.

2. The most recently refined guidelines adopted by the joint panel in 1978 have been closely followed. The emphasis of research has been directed to the projects relating to the pathogenesis and immunological aspects of disease. Subjects of special interest have been immunity and hypersensitivity, the analysis of cell substances of tubercle bacilli which might be related to immunity, and experimental models for the study of tuberculoimmunity. Limited availability of qualified investigators, particularly in mycobacterial genetics, has been a limiting factor.

The panel succeeded in making outstanding progress in the research fields on cellular immunology of tuberculosis and of biologically active mycobacterial components, which contributed significantly to the field of tuberculosis and to other fields, such as general immunology and cancer immunotherapy. It is hoped that future research emphasis will provide greater insight into the field of immunoprophylaxis. The status of BCG vaccination and its duration of efficacy must be clarified. Those factors which delineate natural host immunity to tuberculosis requires elucidation. A correlation to this is a better understanding of various immunologic reactions of tuberculosis patients including anergy. Studies on pathogenesis and immunology of the atypical mycobacterial require emphasis. The panel has adhered to precise and practical objectives aimed at answering the aforementioned problems.

3. The objectives modified in 1978 are appropriate to the specified criteria, but should be subject to periodic scrutiny and revision. New knowledge is being effectively generated by investigators of the Tuberculosis Panel. The "Proceedings" and reports of the joint meetings are circulated and widely quoted by nonparticipants. The panel's activities are pragmatically limited in scope but are complemented in other equally significant areas by leading investigators throughout the world.

One of the historical approaches was the separation and characterization of antigens from tuberculosis culture filtrates by the most sophisticated techniques available. A targeted objective was to obtain highly specific diagnostic reagents. This objective has been disappointing and elusive with the exception of the MAIS glycolipds. Other limiting factors were variations in heterogenity in size and the presence of multiple determinants on individual antigen molecules—consisting of group-specific as well as species-specific epitopes.

In the panel's judgment, this aspect of the program should be redirected since the most sophisticated physical separation processes have not yielded desired results. Now it is necessary to apply monoclonal antibody techniques in order to sharpen methods of antigen isolation and identification. Monoclonal antibodies elicited by individual components with a **specific** moiety may be useful in differentiating *Mycobacterium* species. If antigens bearing a single specific determinant also carry sensitizing group-specific epitopes, the problem of obtaining highly specific diagnostic (e.g. skin-test) reagents may not occur. Whether complexities of antigenic structure will confuse monoclonal techniques is uncertain, but work should be undertaken.

Application of recombinant DNA techniques may be useful for obtaining abundant supplies of specific antigens. These are much more relevant for pathogens that defy *in vitro* cultivation such as *M. leprae* and *Filariae*. The problem with *M. tuberculosis* is not a lack of antigens, but rather a bewildering galaxy of antigenic species, not one of which has been purified to the point of unique antigenic homogeneity. Greater emphasis upon mycobacterial genetics will most likely contribute immediate benefits rather than recombinant DNA research.

A variant of hybridoma technology which might yield helpful results hinges on successful isolation of an effective *immunogen* from among the group of *M. tuberculosis* antigens. Monoclonal antibody stimulated by a unique *immunogenic* determinant (if antigenic) could be profitably exploited using affinity chromatography in order to isolate the entire spectrum of molecular species bearing that determinant from cell lysates or culture filtrates.

It is highly questionable whether such a unique protective immunogen exists. The decision to undertake such studies requires much discussion.

4. With some exceptions, the panel's objectives are being achieved. Antigenic complexities of mycobacteria impose inordinate difficulties on isolation of unique and specific substances; these obstacles adversely affect the search for nonviable immunogenic fractions. Nevertheless, important new data broadened our fundamental knowledge. Important progress has been made in the cellular and biochemical aspects of the immunology of tuberculosis involving mitogenicity of tuberculin; mechanisms of hypersensitivity; anergy and immunosuppression; immunoglobulin response, especially in convalescence; and nonspecific effector mechanisms.

The taxonomy of mycobacteria has been extended by DNA-DNA hybridization studies. Allocation of additional resources would aid materially in advancing knowledge in mycobacterial genetics through recruitment of skilled, young investigators.

Progressive developments have been made on the constitution and structure of the serologically specific antigens of the *M. avium-intracellulare-scrofulaceum* complex, the recognition of an important role for the *M. tuberculosis* sulfolipids in pathogenesis, and important developments on the modes of action of isoniazid and ethambutol. Coupled with these advances is progress on the biosynthesis of mycolic acids, complete synthesis of some mycolic acids, studies on synthesis and biological activities of cord factor as they relate to structure and to physical-chemical properties, and the expansion of investigations on the biology, physiology, and pathogenic mechanisms of nontuberculosis (atypical) mycobacteria. Except for difficulties in antigen separation, in mycobacterial genetics, and in immunoprophylaxis, the current approaches are yielding much valuable data which should be useful in fulfilling the specified goals.

5. The problem of tuberculosis continues to be of major magnitude, and the work of the panel should not be terminated until its major goals have been achieved. Principal among these is the development of a more effective means of immunization. Consensus holds that this is a target more desirable than development of more effective chemotherapy. Effective chemoprophylaxis should not be discarded. Difficulties in antigen separation have tempered the earlier program enthusiasm regarding an effector immunogen. The reliability of taxonomy and speciation based on new methods developed will be improved. This has been significantly strengthened by chemical studies of lipid antigens. The latter may lead to desirable improvements in diagnosis.

The next 5 or 10 years should see the completion of many specified tasks such as: resolution of the various glycolipid and peptidoglycolipid antigens of a host of mycobacteria; complete understanding of modes of action of a variety of chemotherapeutic agents; considerable progress in understanding natural and acquired drug resistance; and completion of syntheses of many biologically active mycobacterial components. On the other hand, development of an effective immunogen and understanding of host responses specific to tuberculosis remains a formidable task which demands the creative and supplemetal contributions of cellular immunologists, chemists, and molecular biologists. The Tuberculosis Panel is a proper medium to ensure success of these objectives.

Continuation of the Tuberculosis Panel was approved by the Joint Committee. As was mandated in July 1982, the Leprosy and Tuberculosis Panels hold joint annual conferences.

1983

Hepatitis Panel

Panel Reviewers: Dr. Wataru Mori
Dr. Edward W. Hook, Jr.

1. Since hepatitis A, B, and D and non-A, non-B infections continue to be worldwide problems, these investigations are relevant to the U.S.-Japan Cooperative Medical Science Program. The ongoing research projects involve: 1) further characterization of these hepatitis viruses, 2) monitoring the changing epidemiology of diseases caused by these viruses, and 3) evaluation of newly developed vaccines and antiviral drugs.

The widespread availability of diagnostic tests for Hepatitis A Virus (HAV) infection has resulted in a better appreciation of the relative importance of HAV in the etiology of viral hepatitis. This virus accounts for 25%–40% of such disease in the United States but its relative importance is decreasing. HAV has been classified as a picornavirus. Its 7,500 base RNA genome has been molecularly cloned and sequenced. The development of HAV vaccines is in progress. Preliminary attempts to develop a live attenuated vaccine were promising but further attenuation of candidate strains is necessary before they will be suitable for wider testing. Killed vaccines have been developed and are undergoing preliminary clinical testing.

Hepatitis B Virus (HBV) was shown to account for some 25%–40% of clinical hepatitis in the United States and to be on the increase, despite the licensing of an effective and safe killed vaccine in 1982. The application of molecular technology to the study of this virus has strengthened its association with the etiology of hepatocellular carcinoma. Genetically engineered second-generation vaccines prepared in mammalian cells or yeast cells have been developed and show promise of replacing the plasma-derived vaccines currently available. Hepatitis B virus has been classified as a member of a new family of viruses, the Hepadnaviridae. Four similar viruses have been identified in woodchucks, ground squirrels, domestic ducks, and snakes and several of these have been useful in elucidating the natural history and molecular virology of the hepadnaviruses.

The unique relationship between Hepatitis Delta Virus (HDV) and HBV has been further studied. The HDV appears to be a unique human pathogen that is defective and requires coinfection with HBV (which serves as a helper virus) for its replication. The 36nm particle associated with infection was shown to contain a unique antigen, delta antigen, and a small (1.75Kb) putative genome of RNA. HDV was shown to have a worldwide (but spotty) distribution. An exception may be Asia, where very little evidence for HDV infection has been obtained. HDV was found to be associated with up to 50% of fulminant hepatitis previously thought to be caused by HBV and to be responsible for epidemics of severe hepatitis among native populations in South America and illicit drug users in the United States.

Non-A, Non-B Hepatitis (NANB) has continued to be a mystery for hepatitis researchers. The failure to detect an antigen-antibody system or to identify a genome unique to this agent (or agents) has limited progress. Two or more distinct agents of blood-borne NANB are thought to exist, based upon epidemiologic investigations and transmission studies in chimpanzees. The latter studies also permitted the identification of essential lipids in at least some strains of NANB.

Epidemic Non-A, Non-B Hepatitis (ENANB) is presumed to be caused by a virus which was first recognized during the past 5 years. It was shown to exist in southern Asia and probably throughout the Middle East. Its epidemiology was similar to that of HAV—predominantly a water-borne disease with major epidemics separated by endemic disease. Evidence has accumulated that the etiologic agent is a 27nm virus that may be a second serotype of HAV, but attempts to characterize it further have been frustrated by lack of clinical materials, inability to infect tissue cultures with it, and difficulty in transmitting it to laboratory animals.

2. The panel's scientific program has fulfilled many of the objectives listed in the guidelines. During the past 5 years progress has been made in understanding and in controlling infections caused by HAV, HBV, HDV, and NANB viruses.

BEST COPY A

3. The objectives listed in the Hepatitis Panel's guidelines continue to be extremely appropriate. The technology is available to achieve the objectives.

4. The approaches employed during the past 5 years should ensure further progress toward the future achievement of the panel's objectives.

5. It is unlikely that each of the objectives will be reached within the next 5 years. The timeframe wherein the panel's objectives can or should be reached, modified, or terminated should be assessed at the annual meetings of the joint panels.

A recommendation that the Hepatitis Panel continue its active research program will be made by the Subcommittee at the next Joint Committee Meeting.

1985

Environmental Mutagenesis and Carcinogenesis Panel

Panel Reviewers: Dr. Tadao Shimao
Dr. Charles C. J. Carpenter

1. The problems of environmental mutagenesis and carcinogenesis are becoming even more important, as new examples of naturally occurring and industrially produced mutagens and carcinogens are recognized with increasing frequency. The differences in the spectra of neoplastic disease in the United States and Japan offer unique opportunities to determine which environmental factors are responsible for the regional differences in prevalence of human malignant disease. The increasing cooperation of the United States and Japan in a number of industrial endeavors makes it all the more important that the two nations continue to work together in this critical area. The U.S.-Japan panel has succeeded in developing remarkably close cooperation, especially in detecting chemical mutagens and carcinogens, and it represents one of the finest models of scientific collaboration between the two countries. The panel's research remains entirely relevant to the goals of the U.S.-Japan Cooperative Medical Science Program.

2. The panel's scientific program truly fulfills the research objectives which it has identified for itself. Because of the scope of the mandate given to the panel, the research program is necessarily a broad one, but it is the feeling of the reviewers that the joint panel has done a remarkably fine job of developing reasonably concise and practical basic objectives. The wise decision was made to narrow the scope of the research into two major areas including 1) detection of chemical pollutants with mutagenic and carcinogenic activity, and 2) surveillance to determine the occurrence and effects of mutagens and carcinogens in the environment, not only in industrialized populations but also in specific populations with unusual exposures to mutagens or carcinogens, or with exceptionally high prevalences of specific types of neoplasms. Four specific objectives have been delineated in each of the two major areas. It is clear to the reviewers that the panel's scientific program has been carefully designed to achieve its objectives, and the reviewers felt that the panel should be especially congratulated on its rapid advances in the development, refinement, and validation of mutagen assay systems for use in mass screening programs. The reviewers do, however, suggest that even greater efforts be directed toward developing human monitoring systems.

3. The refined guidelines remain entirely appropriate for current needs for the definition of environmental mutagens and carcinogens. The collaboration between Japanese and American scientists has resulted in the development of much broader opportunities for the study of specific environmental mutagens and carcinogens.

4. The approaches currently used by the panel are entirely appropriate to its aims. Although the panel's goals are not susceptible to complete fulfillment (we can never expect the full knowledge of all environmental mutagens and carcinogens), the approaches currently being used seem most appropriate in light of available technologies. Both the Japanese and American panelists have been quick to adopt new scientific technologies to pursue their clearly stated goals.

5. In view of the broad scope of the problem of environmental mutagens and carcinogens, there is a clear cut need for continuation of the work of this panel. As certain specific objectives are achieved, the panel should continue to reorder its priorities, setting new objectives based on both new developments in the environment and the availability of new scientific technologies.

It is the opinion of the reviewers that the work of the current Panel on Mutagenesis and Carcinogenesis represents some of the most important work undertaken by the U.S.-Japan Cooperative Medical Science Program, and continued full support of this program is recommended.

All of the objectives of the panel cannot be reached within the next 5 years. The timeframe wherein the objectives can be reached, and the determination as to when the objectives should be modified, should be reassessed annually at joint panel meetings.

The Subcommittee on Program Review and Planning will recommend to the Joint Committee that the Environmental Mutagenesis and Carcinogenesis Panel be continued and that the revised guidelines be adopted.

1985

CHOLERA PANEL

Drs. Shoji Kuwahara and Nathaniel Pierce, Chairmen
Japan and U.S. Cholera Panels, U.S.-Japan
Cooperative Medical Science Program

Dr. Shoji Kuwahara, Chairman (1980)
(Member 1966-1980)
Professor
Department of Microbiology
Toho University School of Medicine
5-21-16 Omori-nishi
Ota-ku, Tokyo 143 Japan

Dr. Nathaniel F. Pierce, Chairman (1977)
(Member 1972-1976)
Professor of Medicine
Johns Hopkins University
School of Medicine
Baltimore City Hospitals
4940 Eastern Avenue
Baltimore, Maryland 21224

PANEL MEMBERS

Japan

Dr. Masahiro Ohashi (1974)
Chief, Department of Microbiology
Tokyo Metropolitan Research
Laboratory of Public Health
24-1, Hyakunincho 3-chome
Shinjuku-ku, Tokyo 160, Japan

Mr. Nobuya Ohtomo (1978)
Managing Director, Department
of Research and Development
The Chemo-Sero-Therapeutic
Research Institute
668 Okubo, Shimizu-machi
Kumamoto 860, Japan

Dr. Yoshifumi Takeda (1981)
Professor
Department of Bacterial Infection
Institute of Medical Science
Tokyo University
4-6-1, Shirokanedai
Minato-ku, Tokyo 108, Japan

United States

Dr. Richard L. Guerrant (1980)
Professor of Medicine
University of Virginia
School of Medicine
Charlottesville, Virginia 22908

Dr. Harley Moon (1977)
SEA/AR
National Animal Disease Center
Federal Research
U.S.D.A.
Ames, Iowa 50010

Dr. John R. Murphy (1980)
Chief of Biomolecular Medicine
University Hospital
Section of Biomolecular Medicine
Evans 116
75 East Newton Street
Boston, Massachusetts 02118

Dr. Takeshi Yokota (1980-)
Professor
Department of Bacteriology
Juntendo University
School of Medicine
2-1-1, Hongo, Bunkyo-ku
Tokyo 113, Japan

Former Members

Dr. Akira Ghoda (1978-1982)
Professor
Department of Hygenic Science
Kitasato University
1-15-1, Kitasato
Sagamihara 228, Japan

Dr. Kenji Takeya, Chairman (1978-1980,
Member 1965-1977) Deceased
Professor
Department of Bacteriology
School of Medicine
Kyushu University
Fukuoka 812, Japan

Dr. Donald C. Robertson (1984-)
Professor
Department of Microbiology
The University of Kansas
Lawrence, Kansas 66045

Former Members

Dr. John C. Feeley (1978-1984)
Chief, Bacterial Immunology Unit
Bacteriology Section
Microbiology Section
Centers for Disease Control
Atlanta, Georgia 30333

Dr. Rolf Freter (1977-1984)
Professor of Microbiology
Department of Microbiology
University of Michigan
6734 Medical Science Building, II
Ann Arbor, Michigan 48104

Dr. John R. Murphy (1976-1981)
Associate Professor
Department of Microbiology and
Molecular Genetics
Harvard Medical School
Boston, Massachusetts 02115

Japanese Cholera Panel

BROAD GOALS

The Japanese Cholera Panel has carried on joint research with the U.S. Cholera Panel on the following: 1) immunity of cholera, 2) pathophysiology and effective treatment of cholera, 3) bacteriology of *Vibrio cholerae* and related bacteria, 4) diarrheal diseases due to cholera-like enterotoxins, and 5) ecology of cholera and related vibrios. The ultimate objective of this joint research is, needless to say, to decrease the morbidity and mortality of cholera and related diarrheal diseases. For this purpose, studies have been performed in bacteriology and genetics, on structure and function of toxins, in pathology, and in immunology of toxins and various bacterial cellular antigens. These studies have greatly improved the treatment of these diarrheal diseases. During the past two decades, however, a cholera epidemic has spread to various parts of Africa and to countries in the Temperate Zone. Also, Japan has had several outbreaks of cholera.

Therefore, the most important objective of the joint research is to develop an immunogen with immunizing capacity superior to the protection afforded by conventionally killed vaccines. In addition to the search for a vaccine made from bacteria with defective toxigenicity but with intact colonizing ability, studies are underway to test the immunogenicity of the combination of toxoid and somatic antigen.

The occurrence of diarrheal disease in humans cannot be optimally reduced unless research also involves travelers' diarrhea due to enterotoxigenic *E. coli* and diarrhea due to *V. parahaemolyticus*, *Yersinia enterocolitica*, *Campylobacter* and others. Studies are now in progress on the virulence factors and on the pathophysiology of these bacteria.

PROGRESS IN PRIORITY AREAS

Microbiological Studies on *V. Cholerae* and Related Bacteria

The chemical structure of the cell wall lipopolysaccharide (LPS) of bacteria in the family *Vibrionaceae* has been extensively studied. The bacteria in this group have LPS with basically the same structure as that of *Enterobacteriaceae*, but their LPS does not have KDO (2-keto-3-deoxy-octonate) which is an important part in the structure of the core of LPS of the latter. In *V. parahaemolyticus* 06, which does have KDO as the exceptional case, KDO is considered to be in the antigenic side chain. It has also been found that specific amino saccharides, perosamine and quinovosamine, are detected only in the classical cholera vibrio, i.e., *V. cholerae* 01, and that generally, differences in the sugar composition of LPS correspond well to the classification obtained by other methods. Through this research, it has been possible to provide information important for classifying and identifying *V. cholerae* 01.

For serotyping of so-called "NAG" vibrios (*V. cholerae* non-01), investigators in Japan and the United States have so far adopted different systems, thus causing considerable confusion. However, as both countries have now exchanged strains and antisera through their research institutions, comparison of respective types is possible. Thus, it has been made possible to reconcile the two systems.

Lysogenicity of *V. cholerae* has also been reexamined, and in addition to the Kappa type phage described by Takeya et al., a phage called Tau has been found, permitting more detailed phage typing.

For *V. cholerae* and *V. fluvialis*, cellular fatty acid composition has been studied, and it has been found in *V. cholerae* that, whether it is 01 or non-01 type, it contained more hexadecenoic acid than hexadecanoic acid. In addition, it has been found that whereas 3-hydroxylauric acid and trans-9-hexadecenoic acid exist, there is no lauric acid, and that cyclopropane fatty acid is not present. Since *V. fluvialis* contained lauric acid, it can be distinguished from *V. cholerae*. Also, in many vibrios, as in the case of other Gram-negative bacteria, isoprenoid quinone was found.

Studies on biotype, serotype and pathogenicity of *V. fluvialis* have made progress. In regards to *E. coli*, two new colonization factors have been found. It has been shown that for production of colonization factor, cAMP is necessary.

Genetic Studies on *V. Cholerae* and Related Bacteria

Drug resistance has been studied with *V. cholerae* strains recently isolated in various parts in the world. Of a total of 1,381 01 type strains, 2.5% were antibiotic-resistant; of 142 non-01 type strains, 11% were resistant. The resistance pattern was essentially the same and transferable within the genus. It was readily transferable outside the genus to *E. coli*. Transferable resistance against the vibriostatic agent 0/129 was also observed in 1.2%. This was always associated with trimethoprim resistance, and belonged to the C-incompatibility group. The study of drug resistance of other kinds of pathogenic vibrios has been continued.

The whole base sequence of the LT gene of *E. coli* has been determined, and it has been shown to be very similar to that of the cholera toxin gene. With *E. coli* Hfr strain as the donor and *Shigella flexneri* as the recipient, a non-pathogenic Hfr strain of the latter was obtained. When the Hfr *Shigella* strain was crossed with an R-type strain, it was possible to transfer the O-antigen and the ability of the R strain to cause guinea pig keratoconjunctivitis. This finding suggests a possible genetic linkage between O-antigen and pathogenicity.

Ecological and Epidemiological Studies on *V. Cholerae* and Related Bacteria

The incidence of cholera in Japan since 1962 was analyzed. Until 1976, all reported cases except two had been imported. But since 1977, the number of cases suspected of being infected within Japan appears to be increasing. Also, the number of incidents where *V. cholerae* was isolated from regions without any patients with cholera or any known carriers of *V. cholerae* have been increasing. At the same time, contamination of imported food by *V. cholerae* is posing serious problems. The strains isolated from the natural environment or food are, in many cases, nontoxigenic, and an increasing number of them are atypical in their biochemical properties and lysogenicity. It will be necessary to study the origin and pathogenic significance of such vibrios.

Other imported organisms suspected of causing diarrheal disease are *Shigella*, *Salmonella*, non-01 *V. cholerae*, *V. parahaemolyticus*, enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), and *Campylobacter*. Also in overseas countries (Thailand, Burma, Kenya, etc.), investigations have been performed on the distribution of such bacteria. The ability of *V. cholerae* to synthesize cAMP has been increasing year by year, and therefore, a hypothesis has been suggested that there may be some correlation between the ability to synthesize cAMP and changes in adaptability to the environment and the pathogenicity of *V. cholerae*.

Studies on Enterotoxins

Detection and assay of enterotoxins

It has been possible to detect and assay each toxin by means of reverse passive agglutination, with polyclonal or monoclonal antibodies against cholera toxin (CT), and *E. coli* LT and ST adsorbed to RBC or latex particles. In particular, latex agglutination is being widely adopted as a simple and easy procedure. Also, a method has been developed for detection of LT by means of coagglutination with *Staphylococcus*, which possesses a protein on its surface and an anti-toxin against cholera enterotoxin.

A method (Biken test) has been devised for the identification of LT. *E. coli* colonies grown on a special agar medium are covered with a polymyxin B disc, which induces the colonies to release toxin into the medium. Anti-LT or anti-CT serum is added to the preparation. If LT toxin is released, a precipitin band develops in the agar with the addition of the anti-LT serum. This is being accepted worldwide as a simple method for detection of LT. A semifluid medium has also been devised for detection of toxins in combination with the agglutination method mentioned above.

Moreover, a method has been developed for the quantitative analysis of CT and LT, distinguished from each other by the ELISA technique. This method is highly sensitive, and useful for a prompt and differential diagnosis.

Studies on cholera toxin

Progress has been made in research on the activation of adenylate cyclase by CT, and an accessory protein factor necessary for activation has been purified.

Direct evidence has been obtained that the entrance of CT into cells occurs not by endocytosis but by penetration through the membrane. Also, it has been demonstrated that not only subunit A but also subunit B enters into cells. With regard to subunit B, concurrent intake of the receptor into cells in the manner of so-called "down regulation" has been observed.

Numbers of monoclonal antibodies against CT have been prepared, and by comparison of their activities for neutralization and binding, the molecular structure and function of CT are being analyzed.

In regards to the production of CT, intracellular localization of CT in *V. cholerae* has been observed by the enzyme-labeled antibody technique. It has been suggested that cAMP is not required for production of CT and that the anaerobic condition of the culture medium has some relation with production of CT. At the same time, it has also been reported that other types of toxin require cAMP for production, but this induces CHO cell elongation without an elevation of intracellular level of cAMP.

Production of enterotoxin has been compared between 01 type and non-01 type of *V. cholerae*. Production rate was highest in 01 classical, followed by 01 El Tor, and then by non-01 type. The production tended to be consistently high in classical type strains. The toxin produced by non-01 *V. cholerae* has been shown to be closely related with 01 toxin (CT). Among the non-01 toxins, however, some are totally indistinguishable from cholera enterotoxin, and others show somewhat different characteristics. Further, there were many non-01 strains that produced a cytotoxic factor.

It was found that CT conjugated with either pullulan or LPS has low toxicity but persistent antigenicity, and, therefore, can be used as an immunogen.

Studies on E. coli. *enterotoxins*

As mentioned above, the amino acid base of the LT gene has been sequenced. As the result of this finding, the configuration of peptide chains in the molecules has been hypothesized, and from this structure a model has been made to show the entrance of the toxin molecule into the cell. The hydrophobic segment of subunit A penetrates the cell membrane after a cellular binding of the B subunit.

As in the case of CT, the structure and functions of LT are being clarified by means of monoclonal antibody and other techniques. LTs produced by *E. coli* of human and porcine origin are considered to be different from each other in their structures, and they are called LTh and LTp, respectively. By comparison with CT, they are shown to have their own specific antigen determinant and common antigen determinant.

STa, namely suckling mouse test-positive ST, also has two types, STh and STp. They have been shown to be peptides comprising 19 and 18 amino acids, respectively. Furthermore, peptides with the same primary structure and biologic activity have been artificially synthesized together with some analogous peptides. From the result of comparative studies on these peptides, the biologically active portion of the ST molecule has been determined.

Other toxins

ST of the *Yersinia enterocolitica* origin was purified. This is a peptide consisting of 30 amino acids, and the 10 amino acid residues at C-terminus were identical with those of ST of *E. coli*. The toxin of *Shigella dysenteriae* was purified, and it was found to be composed of two types of subunits, namely subunit A (ca. 30,000 daltons) and subunit B (ca. 5,000-6,000 daltons) with a molecular weight of ca. 62,000. A similar toxin was obtained from *E. coli*.

Studies on Infection and Immunity by *V. Cholerae* and Related Bacteria

The use of piglets as the model for the experimental infection with *V. cholerae* was proposed. In particular, Cesarean delivered, colostrum-deprived piglets were found to be highly sensitive to *V. cholerae* and CT, and, therefore, were used for studies on pathogenicity of mutant organisms. The sensitivity was about the same in the piglets with intestinal colonization by a few strains of non-pathogenic organisms. Using germ-free suckling mice and intraduodenal inoculation with a tube inserted into the ligated lower ileum of adult rabbits are also proposed. It has been shown that after administration of cimetidine and tincture of opium to adult rabbits, *V. cholerae* colonize the intestine and stimulate immunity. The administration of cimetidine was found useful in facilitating peroral infection in mice.

The aforementioned pullulan- or LPS-conjugated CT can also be used as an oral immunogen. Somatic antigen of *V. cholerae* IF$_{30}$ was studied as an oral immunogen, and was found that oral administration stimulated immunity in mice and suppressed production of IgE. In some cases, tolerance developed to the somatic antigen, depending on the mode of administration of the antigen, but even in the tolerant state, bacteria injected intravenously were effectively eliminated. In an experiment using artificially produced immunodeficient animals, bacterial clearance proved to be mainly due to neutrophils. Also in an experiment with IF$_{30}$, it was found that after peroral immunization, suppressor cells appeared in Peyer's patches. In other experiments, a relationship has been found between the method of immunization and the induction of suppressor cells, i.e., the induction of suppressor cells is very effective after forced feeding with antigen.

When CT is administered to mice, the immune response may be either suppressed or enhanced. This was shown to be related to the reduction and recovery of thymic cells. Also, it has been demonstrated that CT inhibits both the production of cytotoxic lymphocytes and the expression of the lymphocyte effector mechanism.

Pathogenicity of *Shigella* correlates well with its ability to induce keratoconjunctivitis in guinea pigs. It has been found that this is a virulence factor independent of invasiveness into HeLa cells and of 0 antigen. Normal human serum has bactericidal activity against *Shigella*, which is mediated via the classical pathway of complement.

Pathophysiological Studies

When CT was delivered into the intestinal lumen, the alkaline phosphatase in intestinal lymph was first reduced, and then elevated. A similar fluctuation of alkaline phosphatase values was observed in the epithelium of intestinal mucosa. This response was suppressed by the administration of chloropromazine.

The levels of cyclic nucleotides and gastrointestinal hormones in the peripheral blood from patients with acute cholera were examined: pancreatic glucagon was normal in spite of an elevation in total glucagon; VIP and secretin were also normal. There was no constant change in cAMP or cGMP. In animal experiments, somatostatin suppressed hypersecretion due to CT.

Endoscopic and histological examinations of intestinal mucosa were carried out on patients with diarrhea due to enterotoxigenic *E. coli* and non-01 *V. cholerae*. Hyperperistalsis of the gut, and reddening, edema, and hypersecretion by the mucosa were observed in all the cases, but erosion was noted only in severely ill cases.

ACHIEVEMENT OF CHOLERA PANEL DURING THESE 20 YEARS

Bacteriology, Biochemistry, and Genetics

TCBS agar, developed in Japan as a selective medium for isolating vibrios, has remarkably increased the isolation frequency of cholera and related vibrios and made it easier to find cases of cholera. Both biotypes of *V. cholerae* have shown consistent susceptibility to antibacterial agents since 1964. The antibacterial activities of tetracycline and its derivatives are the most effective, followed by chloramphenicol. The antibacterial effectiveness of aminoglycosides and β-lactam antibiotics has been comparatively low.

Since 1960, multiple resistant (R) plasmids transferable in Gram-negative bacteria have been increasing markedly. But many R plasmids are not persistent in *V. cholerae* because of their instability in this bacterium. R plasmids stable in *V. cholerae* have been isolated in Nigeria and other areas since 1977; many of them belong to the C-incompatibility group.

Since 1980, tetracycline-resistant strains have gradually increased in the cholera endemic area, but the frequency of their isolation is much lower than that of tetracycline-resistant organisms of *Enterobacteriaceae*.

Lipopolysaccharides (LPS) of *V. cholerae* and other vibrio species have been purified, and it has been confirmed that, unlike those of *Enterobacteriaceae*, they do not have KDO (2-keto-3-deoxyoctonic acid). Fatty acids with odd numbers of carbons were found only in Inaba type. Cross-reaction of somatic protein antigens was noted between *V. cholerae* and other bacteria such as *Yersinia enterocolitica*.

Research on Cholera Toxin

In 1969, a diarrheagenic substance in culture filtrates of *V. cholerae* was purified by Finkelstein, and was named choleragen. In 1973, Ohtomo et al. and Holmgren showed that choleragen consisted of subunits A and B combining noncovalently with each other. Subunit A is composed of A$_1$ with a molecular weight of ca. 22,000 and A$_2$ with a molecular weight of 5,400. The molecular weight of the subunit B is 11,700. The molecular structures of subunits A and B have now been completely elucidated.

The knowledge of the biological activities of cholera toxin has been markedly increased with improved technology in chemistry and with improved methods for assaying biological activity. Cholera toxin subunit B firstly binds to ganglioside GM$_1$ on the intestinal cell membrane, goes into the double layers of lipid, and the A subunit enters the cytoplasm. Then, A$_1$ activates adenylate cyclase and promotes the secretion of water, sodium, and chloride by the intestinal mucosa via an increase of cyclic AMP, resulting in diarrhea. Cholera toxin is known to suppress cellular immunity, but its effect on the protection against cholera infection is not yet known.

So-called "Non-toxigenic" and "Non-01" *Vibrio Cholerae*

In regards to the serotypes of non-01 *V. cholerae*, it has been possible to correlate the serotypes of Sakazaki and Smith by exchanging bacterial strains and antisera among investigators in Japan and the United States. Among non-01 *V. cholerae* are strains which produce toxins with biological properties similar to those of cholera toxin. However, the physicochemical properties of these toxins are not identical.

The contamination by cholera vibrios which occurred at Tsurumigawa in 1978 emphasized the importance of the environmental contamination problem due to the cholera vibrio. Although the clinical isolates were all pathogenic, vibrios isolated in the environment were mostly nonpathogenic and did not possess toxin genes.

Enterotoxigenic *E. Coli*

Extensive studies have been performed on the toxins produced by enterotoxigenic *E. coli* (ETEC) which is the most common causative organism of so-called "travelers' diarrhea." Among the *E. coli* toxins, the heat-labile toxin (LT) is very similar to cholera toxin in terms of structure and function, and its molecular structure has been determined. Hybrid molecules of both LT and cholera toxin have been prepared. It has furthermore been found that the *E. coli* toxin genes, unlike *V. cholerae*, exist on plasmids, and their gene structure has been elucidated.

When the causative organisms of diarrheal diseases in travelers from various Asian countries were examined, it was found that in most cases the illnesses were due to ETEC. Also, since 1970, the causative organisms of acute enteritis in the Tokyo district have, in many cases, been found to be ETEC.

Some strains of ETEC produce heat-stable toxin (ST) with a smaller molecular weight than that of LT. Such ST is not produced by *V. cholerae*. The molecular structure of ST has been clarified, but is pathogenic mechanism is not as clear as that of LT.

Immunity to Cholera

When cholera toxin was purified, a toxoid detoxified with formalin in the presence of glycine was prepared, and a double blind comparison study was performed between the vaccinated individuals and a nonvaccinated control group in the Phillippines in 1975. However, a satisfactory protective effect was not observed. Somatic antigen IF_{30} (extract from El Tor cholera vibrio V86 strain) was found to have a preventive effect against infection following its oral administration to mice, but humans have not yet been immunized with this antigen.

In order to provide protective immunity against clinical infection, the antigen should be administered orally so that local immunization may be achieved in the intestinal tract. The Texas Star-SR strain of *V. cholerae*, developed by Honda et al., is a mutant strain which produces only the toxin subunit B, having lost ability to produce subunit A; this strain has been found to cause mild diarrhea occasionally in volunteers. However, when virulent *V. cholerae* strains were administered to those volunteers, about 60% protection against cholera was observed. At present, the Texas Star-SR strain is being compared with other types of immunogens such as toxoid/organism combinations.

FUTURE GOALS

The objective which we should urgently pursue is to develop a new type of cholera vaccine. Development of a more effective vaccine against cholera might also lead to the development of an effective control method for travelers' diarrhea due to enterotoxigenic *E. coli*, and, moreover, might bring about marked progress in the research on other kinds of bacterial diarrhea.

Several new species of vibrios with different pathological effects were discovered during taxonomic studies on vibrios. In the future, it will be necessary to expand the scope of bacteriological and pathological research on various NAG vibrios. This research will enable us to analyze, in more detail, various pathological patterns of diarrheal diseases and, consequently, will provide us with basic data for further progress in the treatment and prevention of these diseases.

Pathogenic *E. coli*, *Salmonella*, *Shigella*, *Yersinia enterocolitica*, *Campylobacter*, and some species of clostridia cause diarrhea due to infections or diarrhogenic toxins with different pathogenetic mechanisms; therefore, comparative research on the course of infections, and on the structure and mode of action of toxins, will become an important theme for the panel.

The patterns of occurrence of cholera and similar diarrheal diseases are changing year by year, and the cholera epidemic is gradually extending even to the Temperate Zone. Therefore, epidemiological studies of these diseases is very important for prophylactic countermeasures. It will be necessary to investigate the case incidence, and the routes of dissemination of cholera, in comparison with other diarrheal diseases. It will be necessary simultaneously to pursue research on food vectors, particularly on food imported from places where diarrheal diseases are endemic, as well as on the ecology of vibrios and other causative organisms of diarrhea.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
Nobuya Ohtomo and Toyoharu Muraoka The Chemo-Sero-Therapeutic Research Institute, Kumamoto	Experimental cholera in hysterectomy-produced colostrum-derived (HPCD) piglets
	The structure-function studies of cholera toxin
	Immunogenicity of cholera toxin detoxified by means of conjugation with pullulan or LPS
	Characterization of monoclonal antibodies against cholera toxin

Makoto Ohashi and Yasuo Kudoh Tokyo Metropolitan Research Laboratory of Public Health, Tokyo	Studies on lysogencity of *Vibrio cholerae 01* Production of enterotoxin and other biologically active substances by *Vibrio cholerae* and allied microorganisms Epidemiological studies on cholera and cholera-like diarrheal diseases Studies on antibiotic susceptibility of *Vibrio cholerae*
Yoshifumi Takeda Research Institute for Microbial Diseases, Osaka University, Osaka, Institute of Medical Sciences, Tokyo University, Tokyo	Isolation, purification, and characterization of heat-labile enterotoxin (LT) produced by enterotoxigenic *E. coli*, and its molecular structure Isolation, purification, and characterization of heat-stable enterotoxin (ST) produced by enterotoxigenic *E. coli*, and its molecular structure Development of simple methods for detecting LT and ST Studies on enterotoxin produced by *Vibrio cholerae* non-01 Studies on serological typing of *Vibrio parahaemolyticus*
Takeshi Yokota, Tatsuo Yamamoto School of Medicine, Juntendo University, Tokyo Shogo Kuwahara School of Medicine, Toho University, Tokyo	Molecular genetics on pathogenic factors of enterotoxigenic *E. coli*
Masaaki Iwanaga Ryukyu University School of Medicine, Naha	Isolation and purification of colonization factor of *Vibrio cholerae 01* Studies on enterotoxin production by *Vibrio cholerae 01* Studies on hemolysins produced by various vibrios and their role in diarrheagenicity
Atsuo Ujiiye Gumma Institute of Public Health, Maebashi	Time responses of intestinal fluid secretion and cyclic AMP levels in experimental cholera
Nakaaki Ohsawa Faculty of Medicine, University of Tokyo, Tokyo	The mode of action of cholera toxin Effects of cholera enterotoxin on the function of rat testicle
Sachio Ogata Kyorin University School of Medicine, Mitaka	Purification and immunological characteristics of cross-reacting protein antigen from *Vibrio cholerae* Experimental cholera in adult mice
Akira Ghoda Department of Hygienic Sciences, Kitasato University, Tokyo	Protective effect of cell component of *Vibrio cholerae*, IF30, against cholera infection and suppression of Ig E production Oral immunization against cholera by cholera vibrio and its cell component
Shogo Sasaki and Tadakatsu Shimamura Tokai University School of Medicine, Isehara	Experimental cholera in germfree mice Detection of heat-labile enterotoxin of *Escherichia coli* by the staphylococcal co-agglutination technique Regulation of cholera toxin production
Kenji Takeya School of Medicine, Kyushu University, Fukuoka	Lysogenicity and other properties of the strains of *Vibrio cholerae 01* isolated from the natural environment
Shoichi Shimotori School of Health Science, Kyshu University, Fukuoka	Filamentous structure of *Vibrio cholerae 01* cells appeared under a specific growth condition and its role in infectivity

Yutaka Zinnaka National Defense Medical College, Tokorozawa	Effects of cholera enterotoxin on immune system The mode of entrance of cholera toxin subunits into cells Protective mechanism in early phase of parenteral infection of vibrios and *Escherichia coli* Mechanism of induction of intestinal immune responses
Masaharu Tsuchiya and Hitoshi Asakura School of Medicine, Keio University, Tokyo	Endoscopic and histopathologic studies on cholera and other diarrheal diseases Pathophysiology of secretory diarrhea experimentally induced in rats Gastrointestinal hormones in cholera and other diarrheal diseases
Tatsuro Naito Institute for Tropical Medicine, Nagasaki University, Nagasaki	Electronmicroscopical observation on *Vibrio cholerae* in the state of toxin production Fundamental studies on the bacteriophages to type *Vibrio cholerae* and applicability of phage typing An experimental cholera in adult rabbits and its application Studies on the purification of bacteriologically active components from *Vibrio cholerae*
Rintaro Nakaya Tokyo Medical and Dental University School of Medicine, Tokyo	Mechanisms of mucous membrane infection by *Shigella*
Kazuhito Hisatsune School of Pharmaceutical Sciences, Josai University, Sakado	Studies on chemical, immunological, pharmacological, and antineoplastic properties of endotoxic(0-antigenic) lipopolysaccharides of *Vibrio cholerae* and related *Vibrionaceae* Chemotaxonomical study on *Vibrionaceae* on the basis of the chemical composition of their endotoxic(0-antigenic) lipopolysaccharides with special emphasis on *Vibrio cholerae* and related pathogenic vibrios Study on *Vibrio cholerae* lipopolysaccharides(LPS)-cholera toxin(CT) conjugates as a new type of cholera vaccine
Toshio Miwatani Research Institute for Microbial Diseases, Osaka University, Suita	Mechanism of bacterial intestinal infection

BIBLIOGRAPHY

Abe, H., T. Kanda, Y. Yanai, S. Hashimoto, R. Ogawa, Y. Miyata, M. Shinbo, K. Harada, T. Tsukamoto, Y. Kinoshita, M. Arita, Y. Takeda, and T. Miwatani. Bacteriological study of travelers' diarrhea (1). Isolation of enteric bacteria from patients with travelers' diarrhea at Osaka Airport Quarantine Station during 1979. *J. Jap. Assoc. Infect. Dis. 55*:679–690, 1981 (text in Japanese).

Abe, H., S. Ichiki, S. Hashimoto, H. Nakano, K. Sato, T. Kanda, Y. Yanai, T. Tsukamoto, Y. Kinoshita, M. Arita, T. Honda, Y. Takeda, and T. Miwatani. Isolation and characterization of enterotoxigenic *Escherichia coli* from patients with travelers' diarrhea in Osaka. *J. Dia. Dis. Res. 2*:83–87, 1984.

Aimoto, S., T. Takao, Y. Shimonishi, S. Hara, T. Takeda, Y. Takeda, and T. Miwatani. Amino-acid sequence of a heat-stable enterotoxin produced by human enterotoxigenic *Escherichia coli. Eur. J. Biochem. 129*:259–263, 1982.

Aimoto, S., H. Watanabe, H. Ikemura, Y. Shimonishi, T. Takeda, Y. Takeda, and T. Miwatani. Synthesis of a highly potent and heat-stable analog of an enterotoxin produced by a human strain of enterotoxigenic *Escherichia coli. Biochem. Biophys. Res. Commun. 112*:320–326, 1983.

Brenner, D.J., B.R. Davis, Y. Kudoh, M. Ohashi, R. Sakazaki, T. Shimada, and H.L. Smith, Jr. Serological comparison of two collections of *Vibrio cholerae* non-01. *J. Clin. Microbiol. 16*:319–323, 1982.

Craig, J.P., K. Yamamoto, T. Takeda, Y. Takeda, and T. Miwatani. Vascular permeability activity of *Escherichia coli* heat-stable enterotoxin. *Infect. Immun. 33*:473–476, 1981.

Craig, J.P., K. Yamamoto, Y. Takeda, and T. Miwatani. Production of cholera-like enterotoxin by a *Vibrio cholerae* non-01 strain isolated from the environment. *Infect. Immun. 34*:90–97, 1981.

Cray, W. C., Jr., E. Tokunaga and N. F. Pierce. Successful colonization and immunization of adult rabbits by oral inoculation with *Vibrio cholerae* 01. *Infect. Immun. 41*:735–741, 1983.

DeMol, P., W. Hemelhof, P. Retore, J.P. Batzler, T. Takeda, T. Miwatani, and Y. Takeda. Detection of heat-stable enterotoxin from *Escherichia coli* by ELISA based on monoclonal antibody. *Lancet ii*:285, 1984.

Ehara, M., and H. Shigeno. Acceleration of polymerization of ADP-ribosylated tubulin by cholera toxin. *Tropical Medicine 24*:155–163, 1982.

Enomoto, K. and D.M. Gill. Cholera toxin activation of adenylate cyclase: roles of nucleoside triphosphates and a macro molecular factor in the ADP-ribosylation of the GTP-dependent regulatory component. *J. Biol. Chem. 255*:1252–1258, 1980.

Enomoto, K. and T. Asakawa. Partial purification and properties of a cytosolic protein factor required for the activation of rat liver adenylate cyclase by cholera toxin. *Biomed. Res. 3*:122–131, 1982.

Enomoto, K. and D.M. Gill. Molecular mechanism of the activation of adenylate cyclase by cholera toxin. In *Topics in Molecular Biology*: Bergen, A.S.V. and G.C.K. Roberts eds. Elsevier Science Publishers, Amsterdam, 151–180, 1983.

Enomoto, K. and T. Asakawa. GTPase activity associates with the inhibitory GTP-binding regulatory component of adenylate cyclase purified from rat brain. *FEBS Letter 171*: 233–239, 1984.

Hemelhof, W., P. Retore, P. DeMol, J.P. Butzler, T. Takeda, T. Miwatani, and Y. Takeda. Production of a monoclonal antibody against heat-stable enterotoxin produced by human strain of enterotoxigenic *Escherichia coli. Lancet i*:1011–1012, 1984.

Higaki, M., T. Honda, Y. Takeda, and T. Miwatani. Detection of *Clostridium difficile* toxin in suckling mice. *FEMS Microbiol. Lett. 12*:351–353, 1981.

Hisatsune, K., and S. Kondo. Lipopolysaccharides of R mutants isolated from *Vibrio cholerae. Biochem. J. 185*:77–81, 1980.

Hisatsune, K., A. Kiuye, and S. Kondo. Sugar composition of O-antigenic lipopolysaccharides isolated from *Vibrio parahaemolyticus. Microbiol. Immunol. 24*: 691–701, 1980.

Hisatsune, K., A. Kiuye, and S. Kondo. A comparative study of the sugar composition of O-antigenic lipopolysaccharides isolated from *Vibrio alginolyticus* and *Vibrio parahaemolyticus. Microbiol. Immunol. 25*:127–136, 1981.

Hisatsune, K., S. Kondo, T. Iguchi, M. Machida, S. Asou, M. Inaguma, and F. Yamamoto. Sugar composition of lipopolysaccharides of family *Vibrionaceae*. Absence of 2-keto-3-deoxyoctonate (KDO) except in *Vibrio parahaemolyticus. Microbiol. Immunol. 26*:649–664, 1982.

Hisatsune, K., S. Kondo, T. Iguchi, M. Machida, S. Asou, M. Inaguma, and F. Yamamoto. Occurrence of uronic acid in lipopolysaccharides of *Vibrionaceae. Microbiol. Immunol. 26*:1133–1138, 1982.

Hisatsune, K. Chemistry of endotoxin. *Rinsyo-to-Saikin 10*:249–260, 1983 (text in Japanese).

Hisatsune, K. Molecular structure of endotoxin. In *Endotoxin*: Homma, Y., S. Iwanaga, M. Niwa, and M. Yoshida eds. Ishiyaku Publ. Co., Tokyo, 62–86, 1983 (text in Japanese).

Hisatsune, K., S. Kondo, T. Iguchi, F. Yamamoto, M. Inaguma, S. Kokubo, and S. Arai. Lipopolysaccharides of the family *Vibrionaceae*. In *Bacterial Endotoxin*: Homma, J.Y.,

S. Kanegasaki, O. Lüderitz, T. Shiba, and O. Westphal eds. Verlag Chemie, Weinheim, 187–201, 1984.

Honda, T., S. Chearskul, Y. Takeda, and T. Miwatani. Immunological methods for detection of Kanagawa phenomenon of *Vibrio parahaemolyticus*. *J. Clin. Microbiol. 11*:600–603, 1980.

Honda, T., S. Taga, Y. Takeda, and T. Miwatani. Modified Elek test for detection of heat-labile enterotoxin of enterotoxigenic *Escherichia coli*. *J. Clin. Microbiol. 13*:1–5, 1981.

Honda, T., Y. Takeda, and T. Miwatani. Isolation of special antibodies which react only with homologous enterotoxins from *Vibrio cholerae* and enterotoxigenic *Escherichia coli*. *Infect. Immun. 34*:333–336, 1981.

Honda, T., T. Tsuji, Y. Takeda, and T. Miwatani. Immunological nonidentity of heat-labile enterotoxins from human and porcine enterotoxigenic *Escherichia coli*. *Infect. Immun. 34*:337–340, 1981.

Honda, T., M. Arita, Y. Takeda, and T. Miwatani. Further evaluation of the Biken test (modified Elek test) for detection of enterotoxigenic *Escherichia coli* producing heat-labile enterotoxin and application of the test to sampling of heat-stable enterotoxin. *J. Clin. Microbiol. 16*:60–62, 1982.

Honda, T., C. Sornchai, Y. Takeda, and T. Miwatani. Immunological detection of the Kanagawa phenomenon of *Vibrio parahaemolyticus* on modified selective media. *J. Clin. Microbiol. 16*:734–736, 1982.

Honda, T., M.M.A. Khan, Y. Takeda, and T. Miwatani. Grouping of enterotoxigenic *Escherichia coli* by hydrophobicity and its relation to hemagglutination and enterotoxin productions. *FEMS Microbiol. Lett. 17*:273–276, 1983.

Honda, T., I. Hernadez, T. Katoh, and T. Miwatani. Stimulation of enterotoxin production of *Clostridium difficile* by antibiotics. *Lancet i*:655, 1983.

Honda, T., R. Samakoses, C. Sornchai, Y. Takeda, and T. Miwatani. Detection of a staphylococcal coagglutination test of heat-labile enterotoxin-producing enterotoxigenic *Escherichia coli*. *J. Clin. Microbiol. 17*:592–595, 1983.

Honda, T., Y. Takeda, T. Miwatani, and N. Nakahara. Failure of antisera to thermostable direct hemolysin and cholera enterotoxin to prevent accumulation of fluid caused by *Vibrio parahaemolyticus*. *J. Infect. Dis. 147*:779, 1983.

Honda, T., M. Arita, and T. Miwatani. Characterization of new hydrophobic pili of human enterotoxigenic *Escherichia coli*: a possible new colonization factor. *Infect. Immun. 43*:959–965, 1984.

Ichinose, Y. and M. Iwanaga. Experimental study on the parenteral use of antibiotics for the treatment of bacterial diarrheal diseases. *Tropical Medicine 25*:11–21, 1983.

Ikemura, H., S. Yoshimura, S. Aimoto, Y. Shimonishi, T. Takeda, Y. Takeda, and T. Miwatani. Synthesis of a heat-stable enterotoxin produced by a human strain of enterotoxigenic *Escherichia coli*. *Chemistry Letters*, pp. 101–104, 1983.

Ikemura, H., S. Yoshimura, S. Aimoto, Y. Shimonishi, S. Hara, T. Takeda, Y. Takeda, and T. Miwatani. Synthesis of a heat-stable enterotoxin (STh) produced by a human strain SK-1 of enterotoxigenic *Escherichia coli*. *Bull. Chem. Soc. Jpn. 57*: 2543–2549, 1984.

Ikemura, H., H. Watanabe, S. Aimoto, Y. Shimonishi, S. Hara, T. Takeda, Y. Takeda, and T. Miwatani. Heat-stable enterotoxin (STh) of human enterotoxigenic *Escherichia coli* (strain SK-1). Structure-activity relationship. *Bull. Chem. Soc. Jpn. 57*:2550–2556, 1984.

Ishibashi, M., Y. Kinoshita, Y. Yanai, H. Abe, Y. Takeda, and T. Miwatani. Analysis of antigens of *Vibrio parahaemolyticus* strains possessing new O- and K-antigens. *Japan. J. Bacteriol. 35*:701–706, 1980 (text in Japanese).

Ishibashi, M., Y. Kinoshita, S. Lam, Y. Takeda, and T. Miwatani. Two new K antigens of *Vibrio parahaemolyticus* found in isolates in Singapore. *Japan. J. Bacteriol. 35*:765–766, 1980 (text in Japanese).

Ishibashi, M., Y. Kinoshita, Y. Yanai, H. Abe, Y. Takeda, and T. Miwatani. A new K antigen of *Vibrio parahaemolyticus* found in isolates from travelers. *Japan J. Bacteriol. 36*:591–592, 1981 (text in Japanese).

Ishibashi, M., Y. Kinoshita, Y. Yanai, H. Abe, Y. Takeda, and T. Miwatani. A new K antigen of *Vibrio parahaemolyticus* found in isolates from travelers to the Philippines. *Japan. J. Bacteriol. 36*:781, 1981 (text in Japanese).

Iwanaga, M., A. Utsunomiya, T. Hayashi, H. Shigeno, S. Watanabe, and T. Naito. Interaction between *Vibrio cholerae* and other microorganisms: with reference to host defence mechanism of mice against cholera. *Tropical Medicine 21*:105–113, 1979.

Iwanaga, M., and T. Naito. Morphological changes of *Vibrio cholerae* during toxin production. *Tropical Medicine 21*:187–196, 1979.

Iwanaga, M., and T. Naito. Toxin production and morphological changes of *Vibrio cholerae* in the medium for inducing pleomorphism. *Tropical Medicine 22*:61–68, 1980.

Iwanaga, M., M. Ehara, and T. Naito. Observation of cholera toxin in *Vibrio cholerae* by using enzyme labeled antibody. *Tropical Medicine 23*:13–21, 1981.

Iwanaga, M., K. Mori, and J.N. Kaviti. The routes of cholera spreading in Kenya. *Tropical Medicine 23*:217–222, 1981.

Iwanaga, M., K. Mori, and J.N. Kaviti. *Vibrio cholerae* 01 isolated in Kenya. *J. Clin. Microbiol. 16*:742–743, 1982.

Iwanaga, M., and Y. Ichinose. An experimental cholera in adult rabbits. A new model for the analysis of diarrheal stool. *Tropical Medicine 25*:1–10, 1983.

Kai, A., S. Matsushita, Y. Kudoh, T. Itoh, S. Sakai, and M. Ohashi. Detection of the colonization factor antigen I and II among *Escherichia coli* isolated from diarrheal cases. *Ann. Rep. Tokyo Metr. Res. Lab. P. H. 32*-1:1–6, 1981 (text in Japanese).

Kai, A., T. Itoh, K. Saito, Y. Yanagawa, M. Inaba, M. Takahashi, S. Matsushita, Y. Kudoh, S. Sakai, and M. Ohashi. Two outbreaks of oyster-associated food poisoning due to enterotoxigenic *Escherichia coli* 027:H7. *Ann. Rep. Tokyo Metr. Res. Lab. P.H. 34*:121–125, 1983 (text in Japanese).

Katz, S.P., T. Shimamura, J.P. Dessaint, D. Braverman, and B.H. Waksman. Mechanisms of action of "lymphocyte-activating factor" (LAF). IV. Differential stimulation of T lymphocytes by induced macrophage enzymes (catheptic carboxypeptidase B and serine proteases). *Cell. Immunol. 56*:68–79, 1980.

Komagata, Y. and A. Ghoda. Production of IgE antibody by somatic components of *Vibrio cholerae*. *Japan. J. Bacteriol. 38*:565–572, 1983 (text in Japanese).

Komagata, Y., and A. Ghoda. Suppression of IgE antibody production by component of *Vibrio cholerae*. *J. J. A. Inf. D. 58*:23–28, 1984 (text in Japanese).

Komagata, Y. Suppressive effects on IgE antibody formation of orally administered living and killed *Vibrio cholerae* cells in mice. *J.J.A.Inf.D. 58*:318–326, 1984 (text in Japanese).

Komagata, Y., A. Ghoda, K. Takahashi, and Y. Kazuno. The influence of mode or oral sensitization with *Vibrio cholerae* whole cells and components on humoral antibody formation against a hapten. *J. Tokyo Women's Med. Coll. 54*:24–31, 1984 (text in Japanese).

Kondo, S., T. Iguchi, and K. Hisatsune. Lypopolysaccharides of Group F, a new group of vibrios. *Biochem. Biophys. Res. Commun. 28*:437–442, 1980.

Kudoh, Y. Laboratory diagnosis and epidemiological survey of enterotoxigenic *Escherichia coli* enteritis. In *Gastrointestinal Infections in Southeast Asia* (IV): Chew, A., M. Yu, M. Ohashi, T. Leng, Y. Kudoh, and S. Lam eds. Southeast Asian Medical Information Center, Tokyo, 87–99, 1982.

Matsumoto, M., M. Tsuda, T. Kusumi, S. Takeda, T. Shimamura, and T. Katsunuma. Enhancement by α-1-antichymotrypsin of antibody response in vivo. *Biochem. Biophys. Res. Commun. 100*:478–482, 1981

Matsumoto, M., M. Yamamura, T. Ohkubo, I. Shimizu, T. Shimamura, and K. Katsunuma. Human α-1-antichymotrypsin enhances primary antibody response in the mouse. *Ann. Immunol. (Inst. Past.) 133*:269–280, 1982.

Matsushita, S., Y. Kudoh, and M. Ohashi. Transferable resistance to the vibriostatic agent 2,4-diamino-6,7-diisopropylpteridine(0/129) in *Vibrio cholerae*. *Microbiol. Immunol. 28*:1159–1162, 1984.

Miura, S., H. Asakura, T. Morishita, T. Hibi, Y. Munakata, K. Kobayashi, and M. Tsuchiya. Changes in intestinal alkaline phosphatase activity in cholera toxin-treated rats. *Gut 23*:507–512, 1982.

Miyano, K., M. Ishibashi, N. Kunita, Y. Takeda, and T. Miwatani. Chemotypes of *Vibrio parahaemolyticus* lipopolysaccharides. *FEMS Microbiol. Lett. 8*:23–28, 1980.

Miyano, K., M. Ishibashi, N. Kunita, Y. Takeda, and T. Miwatani. Identification of amino sugars of *Vibrio parahaemolyticus* lipopolysaccharides. *FEMS Microbiol. Lett. 14*:145–148, 1982.

Miyano, K., M. Ishibashi, N. Kunita, Y. Takeda, and T. Miwatani. Demonstration of the presence of alditols and galacturonic acid in *Vibrio parahaemolyticus* 010 lipopolysaccharide. *FEMS Microbiol. Lett. 20*:225–228, 1983.

Mori, K. Studies on the applicability of the phage typing for *Vibrio cholerae*. Phage typing of *Vibrio cholerae* biovar eltor isolated in Kenya. *Tropical Medicine 25*:51–64, 1983 (text in Japanese).

Morishita, T., H. Asakura, Y. Munakata, M. Tsuchiya, and C. Uylangco. Morphologic study on the jejunal mucosa in human cholera. Endoscopic, light, and electron microscopic approaches. *Stomach and Intestine 18*:381–386, 1983 (text in Japanese).

Morishita, T., S. Miura, A. Yoshioka, K. Kobayashi, T. Hibi, H. Asakura, M. Tsuchiya, and C. Uylangco. Cyclic AMP and gastrointestinal hormones of the peripheral blood, intestinal lymph, and jejunal mucosa in cholera diarrhea. In *Gastrointestinal Function*: Kasuya, Y., M. Tsuchiya, F. Nagao, and Y. Matsuno eds. Excerpta Medica, Amsterdam, 69–84, 1983.

Ohashi, M., T. Terayama, H. Ushioda, Y. Kudoh, M. Tsuno, and S. Sakai. Lysogenicity of the strains of *Vibrio cholerae*, biotype eltor recently isolated in various countries. *Microbiol. Immunol. 25*:613–616, 1981.

Oka, M., S. Tsuru, H. Kitani, and Y. Zinnaka. Suppressive effects of cholera toxin on cell-mediated immunity. 1. In vivo suppression of cytotoxic immunity against allograft. *J. Natl. Def. Med. Coll. 4*:195–202, 1979 (text in Japanese).

Oka, M., N. Shinomiya, J. Anbe, Y. Shigeno, S. Tsuru, and Y. Zinnaka. Suppressive effect of cholerae toxin on cell-mediated immunity. 2. The role of cyclic AMP in the generation of cytotoxic T lymphocyte. *J. Natl. Def. Med. Coll. 6*:99–105, 1981 (text in Japanese).

Okajima, Y., T. Shimamura, A. Ozawa, and S. Sasaki. Molecular properties of cholera enterotoxin. *Tokai J. Exp. Clin. Med. 6*:193–201, 1981.

Okamoto, K., Y. Takeda, and T. Miwatani. Purification of a lethal toxin produced by *Shigella dysenteriae*. *Toxicon 20*: 451–456, 1982.

Okamoto, K., A. Miyama, T. Takeda, Y. Takeda, and T. Miwatani. Cross-neutralization of heat-stable enterotoxin activity of enterotoxigenic *Escherichia coli* and of *Yersinia enterocolitica. FEMS Microbiol. Lett. 16*:85–87. 1983.

Okamura, N., T. Nagai, R. Nakaya, S. Kondo, M. Murakami, and K. Hisatsune. HeLa cell invasiveness and O antigen of *Shigella flexneri* as separate and prerequisite attributes of virulence to evoke keratoconjunctivitis in guinea pigs. *Infect. Immun. 39*:505–513, 1983.

Okamura, N., and R. Nakaya. Mechanisms of infection and manifestation of bacterial enteric diseases. In *Enteric Infection*: Saito, M., R. Nakaya, H. Hashimoto, and Y. Mastubara eds. Itensha, Tokyo, 35–40, 1984 (text in Japanese).

Rowe, B., R. Gross, and Y. Takeda. Serotyping of enterotoxigenic *Escherichia coli* isolated from diarrhoeal travelers from various Asian countries. *FEMS Microbiol Lett. 20*:187-189, 1983.

Shigeno, H., and M. Iwanaga. The pathogenicity of Ubol type eltor vibrio. *J. J. A. Inf. D. 57*:240–251, 1983 (text in Japanese).

Shigeno, H. Fundamental studies on the bacteriophages for typing of *Vibrio cholerae. Tropical Medicine 24*:55–63, 1982 (text in Japanese).

Shimamura, T., K. Ishige, K. Hashimoto, and S. Sasaki. Antigenic competition: Suppression of the response to a first antigen by a second antigen. *Tokai J. Exp. Clin. Med. 4*:237–242, 1979.

Shimamura, T., H. Sasadaira, S. Tsuru, Y. Zinnaka, K. Hashimoto, and S. Sasaki. Cyclic AMP and immune responses: Identification of prostaglandin E-producing cells in mouse spleens. *Tokai J. Exp. Clin. Med. 5*:443–449, 1980.

Shimamura, T., S. Tazume, K. Hashimoto, and S. Sasaki. Experimental cholera in germfree suckling mice. *Infect. Immun. 34*:296–298, 1981.

Shimamura, T., S. Tazume, K. Hashimoto, and S. Sasaki. Protection against cholera in suckling mice born of gnotobiotic mothers monoassociated with *Vibrio cholerae*. In *Recent Advances in Germfree Research*: Sasaki, S. et al. eds. Tokai University Press, Tokyo, 739–740, 1981.

Shimamura, T.,K. Hashimoto, and S. Sasaki. Feedback suppression of the immune response in vivo. I. Immune B cells induce antigen-specific suppressor T cells. *Cell. Immunol. 68*:104–113, 1982.

Shimamura, T., K. Hashimoto, and S. Sasaki. Feedback suppression of the immune response in vivo. II. Involvement of prostaglandins in the generation of suppressor-inducer B lymphocytes. *Cell. Immunol. 69*: 192–195, 1982

Shimamura, T., A. Ozawa, and S. Sasaki. Intestinal bacterial flora and local immunity. *Saishi-Igaku 37*:1079–1084, 1982 (text in Japanese).

Shimamura, T., S. Habu, K. Hashimoto, and S. Sasaki. Feedback suppression of the immune response in vivo. III. Lyt-1$^+$B cells are suppressor-inducer cells. *Cell. Immunol. 83*: 221–224, 1984.

Shimizu, T., Y. Itoh, I. Mifuchi, T. Iguchi, S. Kondo, and K. Hisatsune. Antitumor activity of 2-keto-3-deoxyoctonate-free lipopolysaccharide of *Vibrio anguillarum* in mice. *Gann 74*: 279-284, 1983.

Shimizu, T., E. Matsusaka, T. Masuzawa, Y. Yanagihara, I. Mifuchi, T. Iguchi, S. Kondo, and K. Hisatsune. Mitogenicity and adjuvanticity of lipopolysaccharide from a marine bacterium *Vibrio anguillarum* in mice. *Chem. Pharm. Bull. 32*:4165–4169, 1984.

Soedarmono, P., T. Takeda, A. Matsushiro, K. Yamamoto, Y. Takeda, T. Miwatani, and M. Yoshikawa. Genetic labeling of an ent plasmid that encodes heat-labile enterotoxin of enterotoxigenic *Escherichia coli* with kanamycin resistance transposon. *FEMS Microbiol. Lett. 9*:307–310, 1980.

Takao, T., T. Hitouji, S. Aimoto, Y. Shimonishi, S. Hara, T. Takeda, Y. Takeda, and T. Miwatani. Amino acids sequence of a heat-stable enterotoxin isolated from enterotoxigenic *Escherichia coli* strain 18D. *FEBS Microbiol. Lett. 152*:1–5, 1983.

Takeda, T., T. Honda, Y. Takeda, and T. Miwatani. Failure of chlorpromazine to inhibit fluid accumulation caused by *Escherichia coli* heat-stable enterotoxin in suckling mice. *Infect. Immun. 32*:480–483, 1981.

Takeda, T., Y. Takeda, and T. Miwatani. Genetic labeling of an ent plasmid that encodes heat-stable enterotoxin of enterotoxigenic *Escherichia coli* isolated from patients. *Biken J. 24*:127–135, 1981.

Takeda, T., Y. Takeda, and T. Miwatani. Sporadic cases of enterotoxigenic *Escherichia coli* infection. *J. Jap. Assoc. Infect. Dis. 56*:1160–1163, 1982 (text in Japanese).

Takeda, T., Y. Takeda, and T. Miwatani. Improved method for purification of heat-stable enterotoxin of *Escherichia coli. FEMS Microbiol. Lett. 16*:81–84, 1983.

Takeda, T., M. Kitamura, Y. Takeda, and T. Miwatani. In vitro transfer of ent plasmid from *Escherichia coli* to *Shigella dysenteriae* and *Salmonella. FEMS Microbiol. Lett. 18*:253–256, 1983.

Takeda, T., Y. Takeda, S. Aimoto, T. Takao, H. Ikemura, Y. Shimonishi, and T. Miwatani. Neutralization of activity of two different heat-stable enterotoxins (STh and STp) of enterotoxigenic *Escherichia coli* by homologous and heterologous antisera. *FEMS Microbiol. Lett. 20*:357–359, 1983.

Takeda, Y., T. Honda, S. Taga, and T. Miwatani. In vitro formation of hybrid toxins between subunits of *Escherichia coli* heat-labile enterotoxin and those of cholera toxin. *Infect. Immun. 34*:341–346, 1981.

Takeda, Y., T. Honda, H. Sima, I. Hernandez, A. Chantakit, T. Takeda, and T. Miwatani. Some observations on the Biken test for the detection of enterotoxigenic *Escherichia coli*. *Eur. J. Clin. Microbiol.* *2*:50-51, 1983.

Takeda, Y., T. Honda, H. Sima, T. Tsuji, and T. Miwatani. Analysis of antigenic determinants in cholera enterotoxin and heat-labile enterotoxins from human and porcine enterotoxigenic *Escherichia coli*. *Infect. Immun.* *41*:50-53, 1983.

Takenaka, A., T. Nakamura, R. Mori, H. Shibasaki, S. Kondo, and K. Hisatsune. Further studies on bacterial lipopolysaccharide-induced protection against experimental allergic encephalomyelitis in guinea pigs. *J. Neurol. Sci.* *55*:165-174, 1982.

Tokunaga, E., W.C. Cray, Jr., and N.F. Pierce. Compared colonization and immunizing efficiency of toxigenic (A^+B^-) *Vibrio cholerae* and an A^-B^+ mutant (Texas Star-SR) studied in adult rabbits. *Infect. Immun.* *44*:364-369, 1984.

Tsukamoto, T., Y. Kinoshita, S. Taga, Y. Takeda, and T. Miwatani. Value of passive immune hemolysis for detection of heat-labile enterotoxin produced by enterotoxigenic *Escherichia coli*. *J. Clin. Microbiol.* *12*:768-771, 1980.

Tsuji, T., S. Taga, T. Honda, Y. Takeda, and T. Miwatani. Molecular heterogeneity of heat-labile enterotoxin from human and porcine enterotoxigenic *Escherichia coli*. *Infect. Immun.* *38*:444-448, 1982.

Tsuno, M., K. Ohta, S. Yamada, S. Matsushita, K. Saitoh, T. Itoh, and Y. Kudoh. Occurrence of *Vibrio parahaemolyticus* food poisoning outbreaks and characterization of isolates from food poisoning cases in Tokyo (1978-1982). *Ann. Rep. Tokyo Metr. Res. Lab. P. H.* *34*:15-19, 1983 (text in Japanese).

Tsuru, S., M. Seno, C. Tsuchiya, M. Noritake, K. Wasada, and Y. Zinnaka. Contribution of nonimmune phagocytes to protection of mice against *Vibrio cholerae*. *J.Natl. Def. Med. Coll.* *5*:236-241, 1980 (text in Japanese).

Tsuru, S., K. Nomoto, M. Mitsuyama, Y. Zinnaka, and K. Takeya. Importance of polymorphonuclear leucocytes in protection of mice against *Escherichia coli*. *J. Gen. Microbiol.* *122*: 335-338, 1981.

Tsuru, S., Y. Zinnaka, and K. Nomoto. Decrease in cholera toxin-binding T cells in aged mice and human volunteers. *Int. Archs Allergy Appl. Immun.* *64*:217-221, 1981.

Tsuru, S., K. Nomoto, Y. Zinnaka, and K. Takeya. Effects of cholera toxin on lymphoid system I. Reduction of thymus cells after cholera toxin injection. *J. Clin Lab. Immunol.* *6*:181-183, 1981.

Tsuru, S., Y. Zinnaka, K. Nomoto, and K. Takeya. Effects of cholera toxin on the lymphoid system II. Selective augmentation of delayed foodpad reaction in mice. *J. Clin Lab. Immunol.* *6*:185-192, 1981.

Tsuru, S., M. Matsuguchi, N. Ohtomo, Y. Zinnaka, and K. Takeya. Entrance of cholera enterotoxin subunits into cells. *J. Gen. Microbiol.* *128*:497-502, 1982.

Tsuru, S., K. Nomoto, M. Oka, and Y. Zinnaka. Effects of cholera toxin on the lymphoid system III. In vivo generation of cytotoxic lymphocytes. *J. Clin Lab. Immunol.* *10*:209-214, 1983.

Tsuru, S., K. Nomoto, M. Oka, H. Kitani, Y. Zinnaka, and K. Takeya. Augmented delayed footpad reaction in thymus cell-depleted mice induced by cholera toxoid. *J. Clin. Lab. Immunol.* *11*:37-42, 1983.

Utsunomiya, A., K. Mori, T. Hayashi, M. Iwanaga, T. Naito, Z.W. Gatheru, F. Sang, N. Koske, and J.N. Kaviti. Bacteriological study on the diarrhoeal diseases in Kwale district, Coast Province, Kenya. *Tropical Medicine* *24*:235-252, 1982 (text in Japanese).

Yamamoto, K., Y. Takeda, T. Miwatani, and J.P. Craig. Stimulation by lincomycin of production of cholera-like enterotoxin in *Vibrio cholerae* non-01. *FEMS Microbiol. Lett.* *12*:245-248, 1981.

Yamamoto, K., Y. Takeda, T. Miwatani, and J.P. Craig. Purification and some properties of a non-01 *Vibrio cholerae* enterotoxin that is identical to cholera enterotoxin. *Infect. Immun.* *39*:1128-1135, 1983.

Yamamoto, K., T. Honda, Y. Takeda, and T. Miwatani. Production of increased vascular permeability in rabbits by purified thermostable direct hemolysin from *Vibrio parahaemolyticus*. *J. Infect. Dis.* *148*:1129, 1983.

Yamamoto, K., Y. Takeda, T. Miwatani, and J.P. Craig. Evidence that a non-01 *Vibrio cholerae* produces enterotoxin that is similar but not identical to cholera enterotoxin. *Infect. Immun.* *41*:896-901, 1983.

Yamamoto, K., M. Al-Omani, T. Honda, Y. Takeda, and T. Miwatani. Non-01 *Vibrio cholerae* hemolysin: Purification, partial characterization, and immunological relatedness to eltor hemolysin. *Infect. Immun.* *45*:192-196, 1984.

Yamamoto, T. and T. Yokota. Cloning of deoxyribonucleic acid regions encoding a heat-labile and heat-stable enterotoxin originating from an enterotoxigenic *Escherichia coli* strain of human origin. *J. Bacteriol.* *143*:652-660, 1980.

Yamamoto, T. and T. Yokota. *Escherichia coli* heat-labile enterotoxin genes are flanked by repeated deoxyribonucleic acid sequences. *J. Bacteriol.* *145*:850-860, 1981.

Yamamoto, T., T. Yokota, and A. Kaji. Molecular organization of heat-labile enterotoxin genes originating in *Escherichia coli* of human origin and construction of heat-labile toxoid-producing strains. *J. Bacteriol.* *148*:983-987, 1981.

Yamamoto, T., T. Tamura, T. Yokota, and T. Takano. Overlapping genes in the heat-labile enterotoxin operon originating from *Escherichia coli* human strain. *Mol. Gen. Genet.* *188*:356-359, 1982.

Yamamoto, T., and T. Yokota. Release of heat-labile enterotoxin subunits by *Escherichia coli*. *J. Bacteriol.* *150*:1482-1484, 1982.

Yamamoto, T., T. Tamura, M. Ryoji, A. Kaji, T. Yokota, and T. Takano. Sequence analysis of the heat-labile enterotoxin subunit B gene originating in human enterotoxigenic *Escherichia coli*. *J. Bacteriol. 152*:506–509, 1982.

Yamamoto, T., and T. Yokota. Plasmids of enterotoxigenic *Escherichia coli* H10407: Evidence for two-stable enterotoxin genes and a conjugal transfer system. *J. Bacteriol. 153*: 1352–1360, 1983.

Yamamoto, T., and T. Yokota. Sequence of heat-labile enterotoxin of *Escherichia coli* pathogenic for humans. *J. Bacteriol. 155*:728–733, 1983.

Yamamoto, T., T. Nakazawa, T. Miyata, A. Kaji, and T. Yokota. Evolution and structure of two ADP-ribosylation enterotoxins, *Escherichia coli* heat-labile toxin and cholera toxin. *FEBS Letters. 169*:24–246, 1984.

Yamamoto, T., T. Tamura, and T. Yokota. Primary structure of heat-labile enterotoxin produced by *Escherichia coli* pathogenic for humans. *J. Biol. Chem. 259*:5037–5044, 1984.

Yamamoto, T., and T. Yokota. *Escherichia coli* enterotoxin. Gene and plasmid. *Kagaku-to-seibutsu 22*:297–304, 1984 (text in Japanese).

Yanai, Y., T. Kanda, S. Hashimoto, H. Abe, R. Ogawa, M. Ishibashi, Y. Kinoshita, Y. Takeda, and T. Miwatani. Bacteriological study of traveler's diarrhea. (2) *Vibrio parahaemolyticus* strains isolated from patients with traveler's diarrhea. *J.J.A.Inf. D. 55*:701–708, 1981 (text in Japanese).

Yoh, M., K. Yamamoto, T. Honda, Y. Takeda, and T. Miwatani. Effects of lincomycin and tetracycline on production and properties of enterotoxins of enterotoxigenic *Escherichia coli*. *Infect. Immun. 42*:778–782, 1983.

Yoshimura, S., M. Miki, H. Ikemura, S. Aimoto, Y. Shimonishi, T. Takeda, Y. Takeda, and T. Miwatani. Chemical synthesis of a heat-stable enterotoxin produced by enterotoxigenic *Escherichia coli* strain 18D. *Bull. Chem. Soc. Jpn. 57*:125–133, 1984.

Yoshimura, S., T. Takao, H. Ikemura, S. Aimoto, Y. Shimonishi, S. Hara, T. Takeda, Y. Takeda, and T. Miwatani. Chemical synthesis of fully active and heat-stable fragments of heat-stable enterotoxin of enterotoxigenic *Escherichia coli* strain 18D. *Bull. Chem. Soc. Jpn. 57*:1381–1387, 1984.

Yoshioka, M., H. Asakura, S. Miura, Y. Hamada, T. Morishita, T. Hibi, and M. Tsuchiya. Intestinal secretion: The mechanisms of action of cholera toxin and its effects on intestine. *Saishin-Igaku 38*:2358–2364, 1983 (text in Japanese).

Zinnaka, Y. Recent progress in research of *Vibrio cholerae* 100 years after its discovery. *Japan. J. Bacteriol. 39*:713–731, 1984 (text in Japanese).

Japanese Cholera Panel, U.S.-Japan Cooperative Medical Science Program. *Symposium on Cholera, Gifu, 1980*: Proceedings of the 16th Joint Conference, U.S.-Japan Cooperative Medical Science Program, Cholera Panel. Kuwahara, S., and Y. Zinnaka eds. Toho University, Tokyo, 1981.

Japanese Cholera Panel, U.S.-Japan Cooperative Medical Science Program.

Annual report on cholera study. 1979.

Annual report on cholera study. 1980.

Annual report on cholera study. 1981.

Annual report on cholera study. 1982.

U.S. Cholera Panel

ABBREVIATED SUMMARY OF THE THIRD FIVE-YEAR PERIOD

An outbreak of cholera along the gulf coast of Louisiana emphasized the importance of studies on the environmental ecology of *V. cholerae* 01. Such studies show that *V. cholerae* (not necessarily of the virulent 01 serotype) are routinely found in brackish estuarine waters. The association of *V. cholerae* with shellfish was shown to contribute to the spread of infection in Louisiana.

Basic studies on intestinal mucosal immunity, using cholera toxin as an immunologic probe, emphasized the importance of oral immunization for induction of protective mucosal sIgA responses and demonstrated lasting immunologic memory in the mucosal sIgA system. Orally induced antitoxic immunity was shown to protect dogs against experimental cholera. Other vibrio antigens including the O antigen, flagellum and protease also were shown to play a role in evoking protective immunity. Of considerable importance, volunteers infected experimentally with *V. cholerae* also became highly immune to reinfection for at least 1 year, strongly supporting the notion that immunity to cholera should be induced orally, possibly with live avirulent organisms. With this in mind a mutant of *V. cholerae* that produced the B, but not the A, subunit of cholera toxin (A^-B^+) (and was therefore expected to be avirulent) was developed.

The mechanisms of action of cholera toxin was further defined, being shown to involve NAD-dependent ADP ribosylation of a protein that activated cellular adenylate cyclase, apparently irreversibly. Cholera toxin resembles some mammalian hormones in its subunit structure and mechanisms of action, but lacks the target cell specificity of hormones due to its interaction with ubiquitous GM_1 receptors on cell membranes. Initial studies on the genetic regulation of cholera toxin synthesis were also performed, which were the forerunner of those that would eventually lead to production of toxin gene deletion mutants of *V. cholerae*.

Prior research on *V. cholerae* contributed to a rapid advance in knowledge of enterotoxigenic *E. coli* (ETEC). The LT toxin was purified, and an A-B subunit structure similar to that of cholera toxin was defined. LT was shown to be closely related to cholera toxin, but not immunologically identical. The amino acid sequence of the B subunit of LT was determined. LT was shown to be activated by proteolytic "nicking" of the A subunit, rendering it equal in potency with cholera toxin. *E. coli* ST toxin was also purified (MW ~ 5,000) and shown to be structurally heterogeneous, there being two immunologically related types produced by ETEC that infect humans (STh and STp). ST was shown to act by activation of cellular guanylate cyclase, but to have no effect on adenylate cyclase. The LT gene was cloned and a promoter gene that regulates LT transcription was identified. An *E. coli* strain that produced only the B subunit of LT was developed as a possible live oral vaccine strain.

Mucosal adherence of ETEC was studied. A portion of ETEC strains (that produce both ST and LT) adhere via pilus-like antigens termed CFA I or CFA II. Those are encoded by plasmids that may also carry genes for toxin synthesis. Other adherence antigens are being sought to explain the mucosal adherence of strains that produce neither CFA I or CFA II.

Oral rehydration therapy, for dehydration due to acute diarrhea, originally developed in studies of patients with cholera, was shown to be suitable for all causes of diarrhea in all age groups using a single glucose- or sucrose-based electrolyte solution. This important therapeutic tool is being promoted worldwide for treatment of dehydration due to diarrhea.

PROGRESS IN PRIORITY AREAS

Cholera

At present, control of cholera depends mostly upon effective treatment and, in developed countries, on interruption of transmission by provision of safe water and effective sewage disposal; there is no satisfactory vaccine. The ultimate aim of most current research is to develop improved means of control either by effective immunization or better methods of interrupting transmission.

Vibrio ecology and epidemiology

There is growing evidence that virulent *V. cholerae*, 01 survive for prolonged periods in brackish environmental waters, such as estuaries, especially in association with copepods (small crustaceans), aquatic plants, or shellfish. Such environmental reservoirs may serve as a source of human infections over prolonged periods, as has occurred along the United States Gulf Coast where as single *V. cholerae* strain has caused intermittant outbreaks of cholera for at least 11 years. Nontoxinogenic *V. cholerae*, 01 are also found in environmental waters throughout the world, in both cholera-endemic and -nonendemic areas. Such strains have been shown to lack the gene for production of cholera toxin, and several tested strains have proven nonvirulent in volunteers. However, sporadic episodes of diarrhea associated with some of these 01 and non-01 strains suggest they may have other virulence mechanisms. More information is required on the natural history of *V. cholerae*, 01 in the environment and the role of environmental reservoirs as sources of epidemic or endemic disease. Such information may aid efforts to interrupt the transmission of this infection.

Microbiologic investigations

The mechanism of mucosal colonization by *V. cholerae* remains incompletely defined, but appears to be complex and to depend upon several bacterial features. *V. cholerae* do not produce adhesion-promoting pili, but do produce hemagglutinins (both cell-associated and soluble) that may facilitate colonization. These have been partly characterized, but their role as promoters of colonization, and as possible protective immunogens, is not fully established. Two types of cell-associated hemagglutinins occur, 1) mannose sensitive, and 2) fucose sensitive;

these tend to be produced in a biotype-specific manner. A soluble hemagglutinin produced by both biotypes has intrinsic proteolytic activity which may cause "nicking" of the A_1 subunit of secreted cholera toxin (markedly increasing its toxic activity) and the hydrolysis of mucin and fibronectin (aiding penetration of the intestinal mucus gel), and may also aid adhesion to the mucosal surface. Studies in animals using genetically defined nontoxinogenic mutants of *V. cholerae* (either A^-B^- or A^-B^+) have shown that intact cholera toxin also aids the colonization process significantly. Fully toxinogenic parent strains consistently colonize more efficiently than do their A^-B^- or A^-B^+ mutants. The mechanism of this effect is unknown.

Genetically defined toxin-gene deletion mutants of *V. cholerae* cause mild or moderate diarrhea in an appreciable proportion of volunteers, suggesting that these strains produce other, previously unrecognized, "enterotoxins." The mechanism(s) of this diarrhea has not been identified. It must be clarified if fully safe, live avirulent vaccines are to be developed. Candidate "enterotoxins" include a Shiga-like toxin, shown to be produced in small amounts by many 01 and non-01 *V. cholerae*, and the *V. cholerae* hemolysin, produced by most El Tor strains, and genes for which are also present in classical *V. cholerae*. Whether either of these agents causes the diarrhea associated with nontoxinogenic strains is unknown.

Other virulence mechanisms of *V. cholerae* have also been partially clarified. For example, motility, combined with chemotactic attraction to the mucosal surface, probably contributes significantly to virulence, as demonstrated by experimental studies using nonmotile mutants. Such strains, derived from virulent parents, colonized the mucosa poorly and were only weakly virulent.

Toxicology and Genetics

Major progress has been made in the knowledge of cholera toxin, its mechanism of action, and especially in the genetic control of its synthesis. The complete amino acid sequence of cholera toxin has been determined, revealing only minor differences between toxins produced by classical and El Tor strains. Marked similarity has also been demonstrated between the amino acid sequences for the B subunits of cholera toxin and *E. coli* LT. Knowledge of how cholera toxin acts at the molecular level has advanced, revealing in greater detail the guanyl nucleotide-dependent process by which the A subunit causes ribosylation of an essential protein, which then leads to activation of adenylate cyclase and increased intracellular cyclic AMP.

The genes encoding synthesis of cholera toxin A and B subunits have been mapped and cloned, and their DNA sequence determined; regulatory genes have also been identified. The toxin operon was shown to be duplicated in many strains of *V. cholerae*, especially those of the classical biotype, and to be increased in number in hypertoxinogenic strains. The toxin promoter sequence has been identified and characterized using mutants strains. These studies have led directly to the production of A^-B^- or A^-B^+ gene deletion mutants of *V. cholerae* that have been evaluated as potential live oral vaccine candidates (see below).

Recent studies have shown, both epidemiologically and in volunteers, that susceptibility to cholera is genetically determined, being greatest in persons of O blood type. Animal studies have also shown that sensitivity to the secretion-producing effects of cholera toxin are regulated by the major histocompatibility gene complex.

Immunity and Immunizing Agents

Intestinal immunity to cholera. Studies in animals and humans continue to show that mucosal immunity to cholera and related diseases depends mostly upon secretory mucosal IgA antibodies. Such antibodies are best induced by orally administered vaccines that are absorbed and stimulate intestinal lymphoid tissue. Although this can be done using certain nonliving antigens, there is growing evidence that live bacteria which colonize the intestinal mucosa are particularly efficient immunogens. In animals, it has been shown that live *V. cholerae* are readily "ingested" by phagocytic M cells in the epithelium overlying intestinal Peyer's patches. Bacteria are subsequently deposited among underlying lymphoid cells and carried to submucosal follicles, presumably to initiate a mucosal immune response. In contrast, dead *V. cholerae* are absorbed very inefficiently, if at all, by M cells. This observation accords with findings in animals and volunteers that live *V. cholerae* immunize much more efficiently than do killed bacteria and supports efforts to immunize mucosally using materials that are efficiently absorbed by M cells. Typically, a single small oral dose of live virulent or mutant *V. cholerae* may evoke substantial immunity whereas multiple large doses of nonliving antigens are required to stimulate even partial protection.

Another reason for the immunizing efficiency of live bacteria is that they evoke immune responses to an array of bacterial antigens. Marked protective synergy between antibodies directed against *V. cholerae* LPS and cholera toxin has been described in animals, and it is likely that other antibody combinations which interfere with different steps in pathogenesis also act synergistically, e.g. antitoxin and antiflagellar antibodies. The demonstration of "synergistic immunity" strongly suggests that effective cholera vaccines will contain several antigens, rather than a single highly purified antigen.

Specific immunizing agents. Oral immunization with nonliving antigens has focused on combinations of toxin-derived antigens and killed whole bacteria. Cholera toxin is highly immunogenic by mouth, but causes diarrhea. Procholeragenoid, a heat-induced aggregate of cholera toxin, is minimally toxic and purified B subunit is nontoxic. Both are immunogenic in animals, but B subunit is less so than procholeragenoid, the latter being nearly as immunogenic as intact cholera toxin. Nevertheless, B subunit has been extensively studied in humans and is being used in a current vaccine field trial (see below).

In an attempt to improve the efficiency of nonliving oral antigens, efforts have been made to develop adjuvants which might be given with the vaccine. One material with moderate activity as an oral adjuvant has been identified: a lipoidal amine named avridine. It has also been shown that lipid A of Gram-negative bacteria acts as an adjuvant when linked to oral antigen by the use of liposomes. Further work on the development of practical oral adjuvants is required.

Several live *V. cholerae* candidate vaccines have been developed and tested in animals and volunteers. Texas Star SR is a nitrosoguanidine-induced A^-B^+ mutant which grows rather poorly due to other genetic lesions. In animals, this strain colonized inefficiently and was only partially protective against experimental cholera, even when given in inocula of 10^{10} live bacteria; moreover, it evoked a very modest mucosal antitoxin response. More promising are gene-deletion mutants which lack only genes for toxin or A subunit production, are genetically stable, and are prototrophic. In adult rabbits, these strains colonized and protected against experimental cholera nearly as efficiently as did fully virulent strains; moreover, the A^-B^+ varieties evoked moderate mucosal antitoxin responses.

Studies in volunteers. Research on volunteers has continued to be focused on characterizing immunity to cholera and testing candidate vaccines. An important observation has been that volunteers convalescent from experimental cholera are highly protected against an homologous rechallenge for at least 3 years. This unequivocally demonstrates the lasting nature of enteric immunity to cholera and, thus, strongly supports efforts to reproduce such protection by an oral vaccine. Related studies have shown that infection also causes substantial protection against challenge with the heterologous serotype, although animal studies show that the serotype-specific O antigen is an important immunogen.

Nonliving antigens studied as possible oral vaccines include B subunit or procholeragenoid combined with killed *V. cholerae*. Volunteers given 3 doses of 5 mg B subunit plus 2 X 10^{11} killed *V. cholerae* were significantly protected against challenge with virulent *V. cholerae* (64% protection against clinical diarrhea and 80% reduction in stool volume); the killed bacteria given alone were nearly as protective (56% protection against diarrhea, 57% reduction in stool volume). In a related study, procholeragenoid in sequential doses of 50, 50 and 100 ugs combined with a different whole cell vaccine was less protective, apparently due to poorer immunogenicity of the killed *V. cholerae* that were used. None of these vaccines caused any adverse side effects. A field trial of the B subunit-whole cell vaccine is underway in Bangladesh (see below).

There was a major effort to test candidate live attenuated *V. cholerae* oral vaccines. Three strains have been studied most extensively: Texas Star SR (A^-B^+, chemically induced mutant of El Tor strain Ogawa 3083; JBK 70 (A^-B^- gene-deletion mutant of El Tor strain Inaba N16961); and CVD 101 (A^-B^+ gene-deletion mutant of classical strain Ogawa 395). Some 20% - 50% of volunteers fed 10^5 to 10^{10} viable bacteria of these strains developed transient, mild to moderate diarrhea, sometimes with abdominal discomfort and anorexia. Volunteers fed a single dose of 5 X 10^{10} Texas Star SR developed only modest serum antitoxin titers. When challenged with virulent El Tor *V. cholerae*, Ogawa, they showed about 80% protection against illness, but had only moderate protection against fecal shedding of *V. cholerae*. In a second study, feeding of 10^9 Texas Star SR twice caused only 56% protection against challenge with the heterologous serotype of *V. cholerae*. Although Texas Star SR evoked significant protection, it clearly did not match that evoked by fully virulent strains. Studies with strain JBK-70 were more promising. Volunteers fed 10^8 or 10^{10} bacteria one time, showed nearly 90% protection against illness when challenged with the virulent parenteral strain. Also, they were better protected against fecal excretion of the challenge strain. This demonstrates the greater efficacy of a genetically engineered mutant strain, and the induction of marked protection that was not dependent upon cholera antitoxin. These studies offer considerable promise for development of an effective live oral cholera vaccine provided a mutant strain can be developed that does not cause diarrhea during immunization.

Vaccine field trial. A double-blind controlled vaccine trial is underway in Bangladesh, with support from a number of countries and international agencies. The trial will compare the efficacy of killed *V. cholerae* given alone (2 X 10^{11} bacteria given orally three times at 4-week intervals) with that of the same vaccine combined with 1 mg of purified B subunit. The trial is based on evidence that these vaccines are safe and significantly protective in volunteers. In previous studies they were shown to be immunogenic in adult Bangladeshis. The aims of the trial, which will involve 165,000 persons, are:

1. to determine the extent and duration of protection against cholera offered by each vaccine;
2. to determine whether the combined vaccine protects against disease caused by LT-producing *E. coli*;
3. to determine whether infants nursing immunized mothers are protected against cholera; and
4. to correlate protection with antibody responses in serum and breast milk.

Milk-borne immunity to cholera. Epidemiological studies in Bangladesh have conclusively shown that IgA antibodies to cholera toxin and *V. cholerae* LPS, present in breast milk, significantly protect nursing infants from diarrheal illness due to *V. cholerae*, although they do not prevent asymptomatic infection. Each of the antibodies exerts its protective effect independently of the other. These studies directly support the concept of oral immunization of mothers, to induce IgA antibodies in colostrum and milk, as a means of protecting nursing infants from certain enteric infections.

Enterotoxigenic E. coli

Enterotoxigenic *E. coli* (ETEC) is one of the most important causes of diarrhea among young children in developing countries and among persons who travel from developed to developing countries. Illness is caused by *E. coli* which adhere to the intestinal mucosa and elaborate one or two types of enterotoxins; its pathogenesis thus resembles cholera, in a general way. Present research is aimed at clarifying the mechanisms of mucosal adhesion and toxin action, and in searching for effective approaches to immunization.

Enterotoxins

Heat labile toxin (LT). LTs isolated from *E. coli* pathogenic for humans (LTh) and pigs (LTp) differ slightly with regard to the nucleotide sequence of the LT B subunit gene and the amino acid content of the B subunits (6 amino acid differences out of 203 total). The amino acid sequence of the porcine LT B subunit has been determined and shown to have extensive homology with the B subunit of cholera toxin. The genes encoding LT synthesis (both A and B subunits) have been cloned. This has permitted the construction of plasmids carrying only the LT B subunit gene; such plasmids may prove useful in the development of live oral ETEC vaccines that produce only LT B subunit. It has also allowed preparation of an LT gene probe that can rapidly detect LT production by isolated *E. coli* or presence of LT in the stool, in samples of food or from the environment. This technique was very useful in epidemiological studies of ETEC disease.

Heat stable toxin (ST). Three distinct STs have now been identified. ST_h and ST_p, which cause disease in humans, are closely related antigens, and are included in the terms STa or ST I. A third ST, STb or ST II, is antigenically distinct and causes disease only in pigs. STa causes intestinal secretion by activation of mucosal guanylate cyclase. In contrast, STb appears to act by an independent method which does not involve cyclic nucleotides. Its exact mechanism of action is not known. Genes encoding the production of STa and STb have been cloned and gene probes developed, as for LT B subunit. These have greatly aided epidemiological studies of ST disease by eliminating the need for tedious suckling mouse or porcine intestinal loop assays. The gene probes are still somewhat inconvenient, however, because they utilize a short lived radiolabel. Efforts to develop an ELISA assay to detect ST have shown considerable progress. The main difficulty of producing an antibody to the nonimmunogenic ST molecule has been largely overcome with the availability of polyclonal and monoclonal antibodies. A simple ELISA assay for ST would represent a major diagnostic advance.

Colonization factors. Adherence of ETEC to intestinal mucosa is a critical step in pathogenesis. Models for studying such adherence using human ileal epithelial cells have been developed and should yield valuable information on the adherence process. At least three colonization factor antigens (CFA), which form fimbria on the *E. coli* surface and participate in mucosal attachment, have been identified. They are CFA I, CFA II, and E8775. CFA II has been shown to exhibit antigenic polymorphism. Colonization factors are of particular interest because conceivably they may be important immunogens for prevention of ETEC disease. However, many ETEC strains possess none of these three antigens, presumably adhering by other, yet-to-be-identified mechanisms.

Immunity to ETEC Disease

Only modest progress has been made toward development of an ETEC vaccine. As with cholera, it is presumed the vaccine will have to be a multiantigen material, possibly a live genetically engineered mutant or a combination of mutants. The critical antigens to be delivered are, however, only partly understood. Studies in volunteers have shown that immunity to LT, evoked by challenge with an LT-producing strain, gave insignificant protection against rechallenge with an antigenically unrelated LT-producing strain. Attempts to immunize volunteers orally with purified CFA I or CFA II met with only limited success despite the oral administration of large amounts of antigen. Somewhat more promising is evidence that live *E. coli* bearing only the CFA II antigen (i.e. nontoxinogenic) colonize the intestine and evoke significant resistance to illness caused by a fully virulent CFA II-bearing strain. The possibility that vaccine strains which produce several important colonization factor antigens and the B subunit of LT can be genetically constructed is being explored. It would be very desirable for such strains to also evoke immunity to STa, but there is no present knowledge on how this might be done. Carriers for these antigens might be avirulent *E. coli* or another live vaccine such as Ty21a, the avirulent live oral typhoid vaccine.

ABOVE: Gravid copepod *(Eurytemora affinis)* collected from the Patuxent River, washed free of contaminating bacteria, and then artificially recolonized with *Vibrio cholerae* CA # 1 (mag X 60). BELOW: Egg sac from *Eurytemora affinis* showing attached cells of *Vibrio cholerae* CA 401 (mag X 440).

FUTURE GOALS

Studies on the environmental ecology of *V. cholerae* 01 will continue. A major goal will be to determine how virulent *V. cholerae* 01 survive for years in estuarine waters, e.g. the Gulf of Mexico; other goals are to define the role of environmental sites as (a) interepidemic reservoirs and (b) important sources for periodic reintroduction of *V. cholerae* 01 into susceptible populations. Such information should help define effective ways to interrupt the transmission of infection and, possibly, to prevent the initiation of epidemics.

BEST AVAILABLE COPY

Further studies on the virulence mechanisms of *V. cholerae*, 01 are required. The mechanisms of mucosal adhesion needs to be better understood, and the antigens involved in adhesion must be defined. The role of hemagglutinins of *V. cholerae* 01 in this process need particular attention. The cause of diarrhea in persons fed A^-B^- or A^-B^+ gene-deletion mutants needs to be determined. The question is whether diarrhea results from one or more "enterotoxins" produced by these strains or is it an inevitable result of the colonization process, e.g. a "mechanical" perturbation of mucosal function by the colonizing bacteria? The vibrio hemolysin (cytotoxin) and shiga-like toxin deserve particular attention as candidates for study as unrecognized "enterotoxins" of *V. cholerae* 01. If new enterotoxins are defined, their genetics should be determined and gene deletion mutants developed and tested as live oral vaccines. The results of these studies should greatly aid efforts to develop an effective and safe cholera vaccine.

Efforts to develop a satisfactory cholera vaccine will continue. Candidate vaccines will likely be given orally and consist of either multiantigen nonliving preparations or live avirulent mutants of *V. cholerae* 01. Attempts to improve nonliving vaccines should include (a) the inclusion with whole bacteria or bacterial products of a maximally immunogenic, but safe, toxin derivative, such as procholeragenoid, (b) inclusion of any antigens of proven importance in mucosal colonization, and (c) the possible use of coadministered adjuvants. It will be important to specifically determine the immunizing capacity of other poorly studied antigens of *V. cholerae* 01, such as flagellar sheath protein, hemagglutinins, and other outer membrane proteins. Live nontoxinogenic *V. cholerae* 01 should be developed that do not produce putative toxins, such as shiga-like toxin or hemolysin, by genetic manipulation. Their safety and immunizing efficacy merits evaluation in volunteers. If mucosal adhesion is considered likely to cause mild diarrhea, the immunizing efficacy of large inocula of viable, but nonadherent and nontoxinogenic, *V. cholerae* 01 should also be studied in volunteers. When attractive candidate live or nonliving vaccines are developed, studies should be performed to determine optimal immunizing schedules, the duration of protection which they evoke, the extent of protection against heterologous serotypes and biotypes of *V. cholerae* and against LT-producing strains of *E. coli*. The ideal cholera vaccine should be protective after one or two oral doses, inexpensive, and administered easily under adverse conditions. Heat stability and moisture resistance are obvious requirements.

Studies on basic intestinal immunology, especially those related to induction of secretory IgA responses, should continue. Mechanisms which control the antigen-sampling function of M cells should be defined. Similarly, features of live bacteria or nonliving antigens that affect the efficiency of their uptake by M cells should be determined. It should also be determined whether increased uptake of an antigen by M cells is an important determinant of its mucosal immunizing efficiency. Further studies are also required to identify adjuvants for mucosal immune responses, especially those that are active orally, and to determine their mode of action.

Studies of other enterotoxinogenic bacteria, especially ETEC, will also continue. Of particular importance are studies aimed at further definition of the mechanism of intestinal adhesion of ETEC, especially ST-only strains, with a search for additional adherence-factor antigens. It will also be important to determine whether, ETEC produce unrecognized enterotoxins, such as shiga-like toxin (similar to *V. cholerae* 01), that might be of importance in attempts to develop a safe, live oral ETEC vaccine. Diagnostic assays for use in clinical and field studies concerning ETEC need to be improved. Specifically, a simple ELISA or similar type of assay for ST is required, and toxin gene probes for ST and LT that do not use radiolabels are needed. Attempts to develop an ETEC vaccine will continue. Initial efforts should focus on development and testing of genetically engineered strains of *E. coli* that produce colonization factors (CFA I, CFA II, E8775) and the B subunit of LT. If new colonization antigens are defined, these will also be included. Attempts should also be made to genetically engineer *E. coli* that produce an immunogenic but nontoxic form of ST, so that live vaccines can also evoke immunity to this toxin. Studies should be directed toward determination of the role of specific milk antibodies in protecting against ETEC disease in nursing infants and to determine how such antibodies can best be evoked in nursing mothers. Studies on "enterotoxins" produced by a number of other enteric pathogens will continue, including *Campylobacter jejuni*, *Aeromonas hydrophila*, shigella, salmonella and enteropathogenic *E. coli*. The principal aim is to define these toxins and to establish their role in pathogenesis of, and immunity to, acute diarrhea.

A popular watering spot, boat landing, and frequent source of *Vibrio cholerae* serovar 01 along the Ganges River near the Matlab Hospital.

PROJECTS SUPPORTED BY THE PROGRAM

GRANTS

Investigator and Institution	*Title*
L. Joe Berry University of Texas at Austin	Pathogenesis of campylobacter fetus infections
Harvey J. Brandwein Genetic Diagnostics Corp.	Monoclonal antibodies and immunoassay for *E. coli* toxin
Paul S. Cohen University of Rhode Island	Molecular basis of *E. coli* colonization
Sam T. Donta University of Connecticut Health Center	Modulation of receptor-mediated responses to enterotoxin
Stanley Falkow Stanford University	Virulence determinants of *Escherichia coli*
Richard A. Finkelstein University of Missouri at Columbia	*Escherichia coli* diarrheagenic enterotoxins cholera: Pathogenesis and immunology
Rolf G. Freter University of Michigan	Bacterial adhesion in the microecology of the large gut
Michael D. Gill Tufts University	Mechanism of action of cholera toxin
Neal M. Guentzel University of Texas, San Antonio	Pathogenic vibrio-intestinal mucosal interactions
James B. Kaper University of Maryland at Baltimore	Molecular genetics of *Vibrio parahaemolyticus* hemolysin, development of a live oral cholera vaccine
Arnold S. Kreger Wake Forest University	Toxins of *Vibrio vulnificus*
Chao-Hung Lee Indiana University	Dissemination of bacterial enterotoxin genes
John J. Mekalanos Harvard University	Genetic analysis of toxinogenesis in *Vibrio cholerae*
Alison D. O'Brien U.S. Uniformed Services University of the Health Sciences	Shiga-like toxin of *Escherichia coli*
Stephen H. Richardson Wake Forest University	H-2 gene-modulated cholera enterotoxin response
Donald C. Robertson University of Kansas at Lawrence	Pathogenicity of enterotoxigenic *Escherichia coli*
William R. Romig University of California, Los Angeles	Genetic studies on *Vibrio cholerae*

Richard Sack Johns Hopkins University	Development of live oral *E. coli* vaccine
Patricia L. Shipley Virginia Commonwealth University	Colonization factors of enterotoxigenic *E. coli*
Ronald K. Taylor Harvard University	Genetics of cholera toxin regulation and secretion
Michael R. Thompson University of Cincinnati	*E. coli*/Yersinia enterocolitica stable toxins and receptors
Grace M. Thorne New England Medical Center Hospital	Virulence factors of *E. coli* diarrheagenic for man

CONTRACTS

Myron M. Levine University of Maryland at Baltimore	Facility for study of infectious diseases, vaccines
Richard B. Sack Johns Hopkins University	Establish an infectious enteric disease study center

BIBLIOGRAPHY

Baldini, M.M., J.B. Kaper, M.M. Levine, D.A. Candy, and H.W. Moon. Plasmid mediated adhesion in enteropathogenic *Escherichia coli*. *J. Ped. Gastro. and Nutr. 2*:534–538, 1983.

Barry, W.A., and N.F. Pierce. Protein deprivation causes reversible impairment of the mucosal immune response to cholera toxoid/toxin in rat gut. *Nature. 281*:64–65, 1979.

Barry, W.S., and N.F. Pierce. Impaired mucosal immune response to cholera toxin/toxoid in protein-deprived rats. In *Proceedings of the 14th Joint Conference, U.S.-Japan Cooperative Medical Science Program*: Edited by K. Takeya, and Y. Zinnaka. Published by Japanese Cholera Panel, U.S.-Japan Cooperative Medical Science Program, National Institutes of Health, Tokyo, Japan, 115–123, 1978.

Berry, L.J. Pathogenesis of atoxinogenic strains of Gram negative bacteria—a hypothesis. *Klinische Wochenschrift. 60*:693–695, 1982.

Blake, P.A., D.T. Allegra, J.D. Snyder, T.J. Barrett, L. McFarland, C.T. Caraway, J.C. Feeley, J.P. Craig, J.V. Lee, N.D. Puhr, and R.A. Feldman. Cholera — A possible endemic focus in the United States. *New Engl. J. Med. 302*:305–309, 1980.

Boesman-Finkelstein, M., and R.A. Finkelstein. Protection in rabbits induced by the Texas Star-SR attenuated A^-B^+ mutant candidate live oral cholera vaccine. *Infect. Immun. 36*:221–226, 1982.

Boesman-Finkelstein, M., and R.A. Finkelstein. Sequential purification of lactoferrin, lysozyme, and secretory immunoglobulin A from human milk. *FEBS Letters. 144*:1–5, 1982.

Booth, B.A., C.V. Sciortino, and R.A. Finkelstein. Adhesions of *Vibrio cholerae*. In *Microbial Lectins and Agglutinins*. Edited by D. Mirelman. John Wiley & Sons, New York, 1985, in press.

Booth, B.A., C.V. Sciortino, and R.A. Finkelstein. On the trail of adherence mechanisms in *Vibrio cholerae*: Many scents but not much sense. In *Proceedings of Symposium on Bacterial Vaccines*: September 17–20, 1984. National Institutes of Health. Seminars in Infectious Disease. Thieme-Stratton, Inc., New York, 1985, in press.

Booth, B.A., M. Boesman-Finkelstein, and R.A. Finkelstein. *Vibrio cholerae* HA/protease nicks cholera enterotoxin. *Infect. Immun. 45*:558–560, 1984.

Booth, B.A., M. Boesman-Finkelstein, and R.A. Finkelstein. *Vibrio cholerae* soluble hemagglutinin/protease is a metalloenzyme. *Infect. Immun. 42*:639–644, 1983.

Brandwein, H., A. Deutsch, M. Thompson, and R. Giannella. Production of neutralizing monoclonal antibodies to *E. coli* heat-stable enterotoxin. *Infect. Immun.* (Jan.), 1985, in press.

Brandwein, H., A. Deutsch, M.R. Thompson, and R.A. Giannella. Biologically active monoclonal antibodies to *E. coli* heat-stable enterotoxin. *Infect. Immun.* January, 1985, in press.

Brandwein, H., M. Thompson, and R. Giannella. Production of neutralizing monoclonal antibodies to *E. coli* heat-stable enterotoxin. *Federation Proceedings. 43*:1954(Abs. #3130), 1984.

Brayton, P.R., P.A. West, E. Russek, and R.R. Colwell. New selective plating medium for isolation of *Vibrio culnificus* biogroup I. *J. Clin. Microbiol. 17*:1033–1039, 1983.

Brenner, D.J., B.R. Davis, Y. Kudok, M. Ohashi, R. Sakazaki, T. Shimada, and H.L. Smith, Jr. Serological comparison of two collections of *Vibrio cholerae* non-01. *J. Clin. Microbiol. 16*:319–323, 1982.

Burstyn, D.G., L.A. McNicol, and M.J. Voll. Isolation and characterization of spontaneously arising auxotrophic and Kanagawa phenomenon-negative mutants of *Vibrio parahaemolyticus*. *Infect. Immun. 27*:889–896, 1980.

Clements, J.D., R.J. Yancey, and R.A. Finkelstein. Characterization of the heat-labile enterotoxin of *Escherichia coli*. In *Seminars in Infectious Disease, Vol. IV: Bacterial Vaccines*. Edited by L. Weinstein, and B.N. Fields. Thieme-Stratton Inc., New York, 54–63, 1982.

Clements, J.D., R.J. Yancey, and R.A. Finkelstein. Properties of homogeneous heat-labile enterotoxin from *Escherichia coli*. *Infect. Immun.* 29:91–97, 1980.

Clements, J.D., R.J. Yancey, and R.A. Finkelstein. Pure heat-labile enterotoxin (LT) from *Escherichia coli* is similar to choleragen immunologically, structurally, and in specific activity. In *Proceedings of the 15th Joint Conference on Cholera*: Bethesda, Maryland, July 23–25, 1979. U.S. DHEW Pub. No. 80-2003, National Institutes of Health, Washington, D.C., 27–43, 1980.

Clements, M.L., M.M. Levine, C.R. Young, R.E. Black, Y.L. Lim, R.M. Robins-Browne, J.P. Craig. Magnitude, kinetics, and duration of vibriocidal antibody responses in North Americans following ingestion of *Vibrio cholerae*. *J. Infect. Dis. 145*:465–473, 1981.

Colwell, R.R., F.L. Singleton, A. Huq, H.S. Xu, and N. Roberts. Ecology of *Vibrio cholerae*, *Vibrio parahaemolyticus* and related vibrios in the natural environment. In *Proceedings of the International Symposium on Diarrhoeal Diseases*. Osaka, Japan. Marcel Dekker, New York, 1983, in press.

Colwell, R.R., R.J. Seidler, J. Kaper, S.W. Joseph, S. Garges, H. Lockman, D. Maneval, H. Bradford, N. Roberts, E. Remmers, I. Huq, and A. Hug. Occurrence of *Vibrio cholerae* serotype 01 in Maryland and Louisiana estuaries. *Appl. Environ. Microbiol. 41*:555–558, 1981.

Colwell, R.R., R.J. Seidler, J. Kaper, S.W. Joseph, S. Garges, H.A. Lockman, D. Maneval, H. Bradford, N. Roberts, E. Remmers, I. Huq, and A. Huq. Isolation of *Vibrio cholerae* group 0-1 and related vibrios from Maryland and Louisiana estuaries. *Appl. Environ. Microbiol. 41*:555-558, 1981.

Craig, J.P. A survey of the enterotoxic enteropathies. In *Cholera and Related Diarrheas*. Edited by O. Ouchterlony and J. Holmgren. S. Karger, Basel, 15-25, 1980.

Craig, J.P. Prospects for the development of improved cholera vaccines. In *Proc. Third Nobel Conference, Acute Enteric Infections in Children*, New Prospects for Treatment and Prevention. Edited by T. Holme, J. Holmgren, M. Merson, and R. Mollby. Elsevier/Holland Biomedical, Amsterdam, 461-475, 1981.

Craig, J.P., K. Yamamoto, T. Takeda, Y. Takeda, and T. Miwatani. Vascular permeability activity of *Escherichia coli* heat stable enterotoxin. *Infect. Immun. 33*:473-476, 1981.

Craig, J.P., K. Yamamoto, Y. Takeda, and T. Miwatani. Production of cholera-like enterotoxin by a *Vibrio cholerae* non-01 strain isolated from the environment. *Infect. Immun. 34*:90-97, 1981.

Cray, W.C., E. Tokunaga, and N.F. Pierce. Successful colonization and immunization of adult rabbits by oral inoculation with *Vibrio cholerae* 01. *Infect. Immun. 41*:735-741, 1983.

Davis, B.R., G.R. Fanning, J.M. Madden, A.G. Steigerwalt, H.B. Bradford, Jr., H.L. Smith, Jr., and D.J. Brenner. Characterization of biochemically atypical *Vibrio cholerae* strains and designation of a new pathogenic species, *Vibrio mimicus*. *J. Clin. Microbiol. 14*:631-639, 1981.

Dean, E.A., and R.E. Isaacson. Characterization of a 987P pilus receptor. International Symposium on Neonatal Diarrhea (VIDO), pp. 235-248, 1983.

Dean, E.A., and R.E. Isaacson. *In vitro* adhesion of piliated *Escherichia coli* to small intestinal villous epithelial cells from rabbits and the identification of a soluble 987P pilus receptor-containing fraction. *Infect. Immun. 36*:1192-1198, 1982.

Dean, E.A., and R.E. Isaacson. Location and distribution of a receptor for the 987P pilus of *Escherichia coli* in small intestines. *Infect. Immun. 47*, 1985, in press.

Dean, E.A., and R.E. Isaacson. Purification and characterization of a receptor for the 987P pilus of *Escherichia coli*. *Infect. Immun. 47*, 1985, in press.

DeBoy, J.M., G.M. Thorne, C.F. Deneke, and I.K. Wauchsmuth. Colonization factors and *Escherichia coli* belonging to enterotoxin-associated serotypes. *Current Microbiol. 6*:49-53, 1981.

Deneke, C.F., G.M. Thorne, and S.L. Gorbach. Adhesive pili from enterotoxigenic *Escherichia coli*. *Proceedings of the U.S.-Japan Cooperative Medical Science Program, Fifteenth Joint Meeting*. Bethesda, MD, July 1979. NIH Publication No. 80-2003:61-83, 1980.

Deneke, C.F., G.M. Thorne, and S.L. Gorbach. Adhesive pili from enterotoxigenic *Escherichia coli* pathogenic for humans. *Current Chemotherapy and Infect. Dis.*, Amer. Soc. Microbiol., Washington, D.C., 777-778, 1980.

Deneke, C.F., G.M. Thorne, and S.L. Gorbach. Serogroups of attachment pili of enterotoxigenic *Escherichia coli* isolated from humans. *Infect. Immun. 32*:1254-1260, 1981.

Deneke, C.F., K. McGowan, A.D. Larson, and S.L. Gorbach. Attachment of human and pig (K88) enterotoxigenic *Escherichia coli* strains to either human or porcine small intestinal cells. *Infect. Immun. 45*:522-524, 1984.

Deneke, C.F., K. McGowan, G.M. Thorne, and S.L. Gorbach. Attachment of enterotoxigenic *Escherichia coli* to human intestinal cells. *Infect. Immun. 39*:1102-1106, 1983.

Donta, S.T. Diarrhea in the non-traveler, work-up, and treatment and diarrhea in travelers, causes, prevention, and therapy. In *The Management of Infectious Diseases in Clinical Practice. Part II, Gastrointestinal and Intra-abdominal Infections*. Edited by P.K. Peterson, L.D. Sabath, E. Calderon, Jr., and A.R. Ronald. Academic Press, 61-71, 1982.

Donta, S.T. Effects of monoclonal antibodies to cholera toxin on the binding of cholera and *E. coli* enterotoxins to mammalian cells. *Clin. Res.*, 1983.

Donta, S.T. Modulation of enterotoxin binding to intestine by toxin derivatives. *Clin. Res. 32*:366A, 1984.

Donta, S.T. Receptors on host-cells for microbial toxins. Symposium on Receptors in Human Disease. American Physiological Society, Atlanta, Georgia, May 1981.

Donta, S.T. Regulation of enterotoxin receptors. *Annals, New York Academy of Sciences*, in press.

Donta, S.T., and N.J. Poindexter. Competition for cholera toxin receptors by *Escherichia coli* enterotoxin. In *Current Chemotherapy and Infectious Disease*. Proceedings of the 11th International Congress of Chemotherapy, and the 19th Interscience Conference on Antimicrobial Agents and Chemotherapy. Edited by J.D. Nelson and C. Grassi. Published by American Society for Microbiology, 2:921-922, 1980.

Donta, S.T., and N.J. Poindexter. Detection of cholera toxin production by non-01 *Vibrio cholerae*. American Society for Microbiology, Iowa City, Iowa, October, 1981.

Donta, S.T., and N.J. Poindexter. Regulation of cholera toxin receptors. 17th U.S.-Japan Conference, Baltimore, Maryland, October, 1981.

Donta, S.T., N.J. Poindexter, and B. Ginsberg. Comparison of the binding of cholera and *E. coli* enterotoxins to Y1 adrenal cells. *Biochem. 21*:660-664, 1982.

Dreyfus, L.A., and D.C. Robertson. Solubilization and partial characterization of the intestinal receptor for *Escherichia coli* heat-stable enterotoxin. *Infect. Immun. 46*:537–543, 1984.

Dreyfus, L.A., and D.C. Robertson. Studies on the mechanism of action of the *Escherichia coli* heat-stable enterotoxin (ST_a). In *Advances in Research on Cholerae and Related Diarrheas.* KTK Scientific Publishers, Tokyo, Japan, 269–278, 1983.

Dreyfus, L.A., J.C. Frantz, and D.C. Robertson. Chemical properties of heat-stable enterotoxins produced by enterotoxigenic *Escherichia coli* of different host origins. *Infect. Immun. 42*:539–548, 1983.

Dreyfus, L.A., L. Jaso-Friedmann, and D.C. Robertson. Characterization of the mechanism of action of *Escherichia coli* heat-stable enterotoxin ST_a. *Infect. Immun. 44*:493–501, 1984.

Donta, S.T., N.J. Poindexter, and B. Ginsberg. Comparison of the binding of cholera and *E. coli* enterotoxins to Y1 adrenal cells. *Biochem. 21*:660–664, 1982.

Dreyfus, L.A., and D.C. Robertson. Solubilization and partial characterization of the intestinal receptor for *Escherichia coli* heat-stable enterotoxin. *Infect. Immun. 46*:537–543, 1984.

Dreyfus, L.A., and D.C. Robertson. Studies on the mechanism of action of the *Escherichia coli* heat-stable enterotoxin (ST_a). In *Advances in Research on Cholerae and Related Diarrheas.* KTK Scientific Publishers, Tokyo, Japan, 269–278, 1983.

Dreyfus, L.A., J.C. Frantz, and D.C. Robertson. Chemical properties of heat-stable enterotoxins produced by enterotoxigenic *Escherichia coli* of different host origins. *Infect. Immun. 42*:539–548, 1983.

Dreyfus, L.A., L. Jaso-Friedmann, and D.C. Robertson. Characterization of the mechanism of action of *Escherichia coli* heat-stable enterotoxin ST_a. *Infect. Immun. 44*:493–501, 1984.

Field, L.H., J.L. Underwood, and L.J. Berry. The role of gut flora and animal passage in the colonization of adult mice with *Campylobacter jejuni. J. Med. Micro. 17*:59–66, 1984.

Field, L.H., J.L. Underwood, L.M. Pope, and L.J. Berry. Intestinal colonization of neonatal animals by *Campylobacter fetus* subspecies *jejuni. Infect. Immun. 33*:884–892, 1981.

Finberg, L., P.A. Harper, H.E. Harrison, and R.B. Sack. Oral rehydration for diarrhea. *J. Pediatrics. 101*:497–499, 1982.

Finkelstein, R.A. Antigenic and structural variations in the cholera/coli family of enterotoxins. *Develop. Biol. Standard. 53*:93–95, 1983.

Finkelstein, R.A. Application of monoclonal antibodies and genetically engineered hybrid B subunit proteins to the study of the structure of the cholera toxin family. In *Proceedings of the IX International Symposium on Intestinal Microecology:* September 16–18, 1984. Herborn-Dill, FRG., 1985, in press.

Finkelstein, R.A. Cholera. In *Bacterial Vaccines.* Edited by R. Germanier. Academic Press, Inc., 107–136, 1984.

Finkelstein, R.A. Cholera and related enterotoxins: Tools for membranologists. In *Membranes, Molecules, Toxins, and Cells.* Edited by K. Bloch, L. Bolis, and D.C. Tosteson. PSG Publishing Co., Inc., Boston, 59–68, 1981.

Finkelstein, R.A. Cholera—the great teacher: 100 years since Koch's postulate. In *Proceedings of the Robert Koch Memorial Symposium on Cholera:* November 5, 1984, Kasiwara City, Japan. Martinus Nijhoff Publishers, 1985, in press.

Finkelstein, R.A. Current developments with cholera vaccines: "Where do we go from here?" In *Progress in Clinical and Biological Research, Vol. 47, New Developments With Human and Veterinary Vaccines.* Edited by A. Mizrahi, I. Hertman, M.A. Klingberg, and A. Kohn. Alan R. Liss, Inc., New York, 133–142, 1980.

Finkelstein, R.A. Enterotoxins: Brief historical overview and introduction. *Prog. Fd. Nutr. Sci. 7*:133–138, 1983.

Finkelstein, R.A. Immunology of *Vibrio cholerae.* In *Comprehensive Immunology (series): Immunology of Human Infection.* Edited by A.J. Nahmias, and R.J. O'Reilly. Plenum Publishing Corp., New York, 291–315, 1981.

Finkelstein, R.A. Laboratory production and isolation of enterotoxins and isolation of a candidate lie vaccine for diarrheal disease. In *Proceedings, 43rd Nobel Symposium: Cholera and Related Diarrheas--Molecular Aspects of a Global Health Problem.* August 7–11, 1978, Stockholm, Sweden. S. Karger, Basel, Switzerland, 64–79, 1980.

Finkelstein, R.A. Structure of the cholera enterotoxin (choleragen) and the immunologically related ADP-ribosylating heat-labile enterotoxins. In *Handbook of Natural Toxins, Vol. II: Bacterial Toxins.* Edited by M.C. Hardegree, W.H. Habig, and A. Tu. Marcel Dekker, Inc., 1984, in press.

Finkelstein, R.A. The cholera/coli family of enterotoxins. *Toxicon. 3(Suppl.)*:145–147, 1983.

Finkelstein, R.A. *Vibrio cholerae* (and other pathogenic vibrios). In *Medical Microbiology: Principles and Concepts.* Edited by S. Baron. Addison-Wesley Publishing Co., 333–343, 1982.

Finkelstein, R.A. *Vibrio cholerae* (and other pathogenic vibrios). In *Medical Microbiology, 2nd Edition: Principles and Concepts.* Edited by S. Baron. Addison-Wesley Publishing Co., 1984, in press.

Finkelstein, R.A., and F. Dorner. Cholera enterotoxin (choleragen). In *The International Encyclopedia of Pharmacology and Therapeutics, "Pharmacology of Bacterial Toxins."* Edited by F. Dorner. 1984, in press.

Finkelstein, R.A., and L.F. Hanne. Hemagglutinins (colonization factors?) produced by *Vibrio cholerae. Microbiology. 1982*:324–326, 1982.

Finkelstein, R.A., and L.F. Hanne. Purification and characterization of the soluble hemagglutinin ("cholera lectin") produced by *Vibrio cholerae*. *Infect. Immun. 36*:1199–1208, 1982.

Finkelstein, R.A., and M. Boesman-Finkelstein. Observations on the Texas Star-SR avirulent A^-B^+ mutant candidate cholera vaccine. *Develop. Biol. Standard. 53*:89–92, 1983.

Finkelstein, R.A., and Z. Yang. Rapid test for identification of heat-labile enterotoxin-producing *Escherichia coli* colonies. *J. Clin. Microbiol. 18*:23–28, 1983.

Finkelstein, R.A., B.A. Marchlewicz, R.J. McDonald, and M. Boesman-Finkelstein. Isolation and characterization of a cholera-related enterotoxin from *Salmonella typhimurium*. *FEMS Microbiology Letters. 17*:239–241, 1983.

Finkelstein, R.A., C.V. Sciortino, L.C. Rieke, M.F. Burks, and M. Boesman-Finkelstein. Preparation of "procoligenoids" from *Escherichia coli* heat-labile enterotoxins (LTs). *Infect. Immun. 45*:518–521, 1984.

Finkelstein, R.A., M. Boesman-Finkelstein, and P. Holt. *Vibrio cholerae* hemagglutinin/lectin/protease hydrolyzes fibronectin and ovomucin: F.M. Burnet revisited. *Proc. Natl. Acad. Sci. 80*:1092-1095, 1983.

Finkelstein, R.A., M. Boesman-Finkelstein, and R.K. Watson. The Texas Star-SR attenuated A^-B^+ mutant candidate live oral vaccine against cholera (and related enterotoxic enteropathies). In *Proceedings of the Conference on Experimental Cholera Vaccines*. International Centre for Diarrhoeal Disease Research, Bangladesh. Special Publication No. 15, 72–88, 1981.

Finkelstein, R.A., M.F. Burks, L.C. Rieke, R.J. McDonald, S.K. Browne, and W.S. Dallas. Application of monoclonal antibodies and genetically-engineered hybrid B-subunit proteins to the analysis of the cholera/coli enterotoxin family. *Develop. Biol. Standard.*, 1984, in press.

Finkelstein, R.A., M.F. Burks, L.C. Rieke, R.J. McDonald, S.K. Browne, and W.S. Dallas. Monoclonal antibody analyses of the cholera-related enterotoxin family. In *Advances in Research on Cholera and Related Diarrheas*. KTK Scientific Publishers, Tokyo, Japan, 1984, in press.

Finkelstein, R.A., Z. Yang, S.L. Moseley, and H.W. Moon. Rapid latex particle agglutination test (LPAT) for heat-labile enterotoxin-producing *Escherichia coli* strains of porcine origin. *J. Clin. Microbiol. 18*:1417–1418, 1983.

Frantz, J.C., and D.C. Robertson. High affinity receptor for heat-stable enterotoxins (ST_a) on rat intestinal epithelial cells. In *Advances in Research on Cholerae and Related Diarrheas*. KTK Scientific Publishers, Tokyo, Japan, 247–257, 1983.

Frantz, J.C., and D.C. Robertson. Immunological properties of *Escherichia coli* heat-stable enterotoxins: Development of a radioimmunoassay specific for heat-stable enterotoxins with suckling mouse activity. *Infect. Immun. 33*:193–198, 1981.

Frantz, J.C., L. Jaso-Friedmann, and D.C. Robertson. Binding of *Escherichia coli* heat-stable enterotoxin to rat intestinal cells and brush border membranes. *Infect. Immun. 43*:622–630, 1984.

Geary, S.J., B.A. Marchlewicz, and R.A. Finkelstein. Comparisons of heat-labile enterotoxins from porcine and human strains of *Escherichia coli*. *Infect. Immun. 36*:215–220, 1982.

Giannella, R.A., M. Luttrell, and M.R. Thompson. Binding of *E. coli* heat-stable enterotoxin to receptors on rat intestinal cells. *Am. J. Physiol. 245*:G492–G498, 1983.

Giannella, R.A. M.R. Thompson, and H. Brandwein. Monoclonal antibodies to *Escherichia coli* heat-stable enterotoxin. *Lancet. 1*:1360, 1984.

Gill, D.M., and S.H. Richardson. Adenosine diphosphate-ribosylation of adenylate cyclase catalysed by heat-labile enterotoxin of *Escherichia coli*: Comparison with cholera toxin. *J. Infect. Dis. 141*:64–70, 1980.

Gill, D.M., and S.H. Richardson. ADP-ribosylation of adenylate cyclase catalysed by the heat-labile enterotoxin of *Escherichia coli*. In *Proceedings of the Joint U.S.-Japan Conference on Cholera and Related Diseases*. National Institutes of Health Publication No. 80-2003, 142–147, 1980.

Gill, D.M., J.D. Clements, D.C. Robertson, and R.A. Finkelstein. Subunit number and arrangement in *Escherichia coli* heat-labile enterotoxin. *Infect. Immun. 33*:677–682, 1981.

Gilligan, P.H., J.C. Brown, and D.C. Robertson. Immunological relationships between cholera toxin and *Escherichia coli* heat-labile enterotoxin. *Infect. Immun. 42*:683–691, 1983.

Greenberg, R.N., F. Murad, B. Chang, D.C. Robertson, and R.L. Guerrant. Inhibition of *Escherichia coli* heat-stable enterotoxin by indomethacin and chlorpromazine. *Infect. Immun. 29*:908–913, 1980.

Greenberg, R.N., R.L. Guerrant, B. Chang, D.C. Robertson, and F. Murad. Inhibition of *Escherichia coli* heat-stable enterotoxin effects on intestinal guanylate cyclase and fluid secretion by quinacrine. *Biochem. Pharmacol. 31*:2005–2009, 1982.

Grimes, D.J., J. Stemmler, H. Hada, E.B. May, D. Maneval, F.M. Hetrick, R.T. Jones, M. Stoskopf, and R.R. Colwell. *Vibrio* species associated with mortality of sharks held in captivity. *Microb. Ecol. 10*:271–282, 1984.

Grimes, D.J., R.R. Colwell, J. Stemmler, H. Hada, D. Maneval, F.M. Hetrick, E.B. May, R.T. Jones, and M. Stoskopf. *Vibrio* species as agents of elasmobranch disease. *Helgolander Meeresunters. 38*,1984, in press.

Guentzel, M.N., B. Hinman, R. Bell, T.V. Gay, and D. Guerrero. The role of mucin in the pathogenesis of *Vibrio cholerae*. Annual Meeting ASM. *21*:abstract, 1980.

Guentzel, M.N., D. Amerine, D. Guerrero, and T.V. Gay. Association of *Vibrio cholerae* mutants with the intestinal mucosa of infant mice. *Scanning Electron Microsc. 4*:115-124, 1981.

Guentzel, M.N., D. Guerrero, and R. DeShambo. Nature of interaction of different wild type *Vibrio cholerae* with intestinal mucosa. *Scanning Electron Microsc. 1*:263-270, 1983.

Guerrant, R.L., J.M. Hughes, B. Chang, D.C. Robertson, and F. Murad. Activation of intestinal guanylate cyclase by heat-stable enterotoxin of *Escherichia coli*: Studies of tissue specificity, potential receptors and intermediates. *J. Infect. Dis. 142*:220-228, 1980.

Hada, H.A., J. Stemmler, P.A. West, and R.R. Colwell. Molecular genetic studies of luminescent *Vibrio cholerae* serovar non-01 isolated from the aquatic environment. Abstract, 1982 Annual Meeting, Canadian Society for Microbiologists, Quebec, Canada, 1982.

Hada, H.S., J. Stemmler, M.L. Grossbard, P.A. West, C.J. Potrikus, J.W. Hastings, and R.R. Colwell. Characterization of non-01 *Vibrio cholerae (Vibrio albensis). Syst. Appl. Microbiol.* 1985, in press.

Hanne, L.F., and R.A. Finkelstein. Characterization and distribution of the hemagglutinins produced by *Vibrio cholerae. Infect. Immun. 36*:209-214, 1982.

Hirst, T.R., J. Sanchez, J.B. Kaper, S.J.S. Hardy, and J. Holmgren. Mechanism of toxin secretion of *Vibrio cholerae* investigated in strains harboring plasmids that encode *Escherichia coli's* heat-labile enterotoxins. *Proc. Natl. Acad. Sci. USA*, 1985, in press.

Honda, T., and R.A. Finkelstein. Isolation of a novel *Vibrio cholerae* mutant potentially suitable for use as a live vaccine against cholera and related diarrheas. In *Proceedings of the 15th Joint Conference on Cholera:* Bethesda, Maryland, July 23-25, 1979. U.S. DHEW Pub. No. 80-2003, National Institutes of Health, Washington, D.C., 94-99, 1980.

Huq, A., E.G. Small, P.A. West, M.I. Hug, R. Rahman, and R.R. Colwell. Ecological relationships of *Vibrio cholerae* with planktonic crustacean copepods. *Appl. Environ. Microbiol. 45*:275-283, 1983.

Isaacson, R.E. Bacterial adherence to mucosal surfaces: An attribute of virulence. *Bull. European Physiopathologie Respiratoire. 19*:75-80, 1983.

Isaacson, R.E. Factors affecting expression of the *Escherichia coli* pilus, K99. *Infect. Immun. 28*:190-194, 1980.

Isaacson, R.E. Mechanisms of adhesion: Animal cells (pili). In *Bacterial Adhesion: Mechanisms and Physiological Significance*. Edited by D. Savage, and M. Fletcher. Plenum Press.

Isaacson, R.E. Pili of enterotoxigenic *Escherichia coli*. International Symposium on Neonatal Diarrhea (VIDO). p.p. 213-236, 1980.

Isaacson, R.E. Pili of enterotoxigenic *Escherichia coli* of calves and pigs. In *Proceedings of the Third International Symposium on Immunobiology of Proteins and Peptides*. Edited by M.Z. Atassi. Plenum Press, 1984.

Isaacson, R.E. Regulation of pilus expression. In *Attachment of Organisms to the Gut Mucosa*. Edited by E.C. Boedeker. CRC Press, 121-128, 1984.

Isaacson, R.E. Regulation of the *Escherichia coli* pilus K99. *Infect. Immun. 40*:633-639, 1983.

Isaacson, R.E., and P. Richter. *Escherichia coli* 987P pilus: Purification and partial characterization. *J. Bacteriol. 146*:784-789, 1981.

Isaacson, R.E., and P. Richter. Molecular and genetic analysis of K99 plasmids. International Symposium on Neonatal Diarrhea (VIDO). p.p. 179-190, 1983.

Isaacson, R.E., E.A. Dean, R.L. Morgan, and H.W. Moon. Immunization of suckling pigs against enterotoxigenic *Escherichia coli*-induced diarrheal disease by vaccinating dams with purified K99 or 987P pili: Antibody production in response to vaccination. *Infect. Immun. 29*:824-826, 1980.

Isaacson, R.E., J. Colmenero, and P. Richter. *Escherichia coli* K99 pili are composed of one subunit species. *FEMS Microbiol. Lett. 12*:229-232, 1981.

Isaki, L.S., R.A. Bever, J.W. Newland, and M.J. Voll. Isolation and characterization of small plasmids in strains of *Vibrio parahaemolyticus. J. Diar. Dis. Res. 1*:99-106, 1983.

Jones, G.W., and R.E. Isaacson. Proteinaecous bacterial adhesins. In *Critical Reviews in Microbiology*. CRC Press, *10*:229-260, 1983.

Joseph, S.W., J.B. Kaper, and R.R. Colwell. *Vibrio parahaemolyticus. CRC Crit. Rev. Microbiol. 10*:77-124, 1982.

Joseph, S.W., S.T. Donta, D.R. Maneval, J.B. Kaper, R.R. Colwell, and W.M. Spira. An assessment of non-01 *Vibrio cholerae* virulence in the Y-1 mouse adrenal cell assay. In *Vibrios in the Environment*. Edited by R.R. Colwell. J. Wiley and Sons, New York, 123-143, 1984.

Kaper, J.B. Molecular toxigenic characterization of environment *V. cholerae*. In *Vibrios in the Environment*. Wiley and Sons, Inc., New York, 239-247, 1984.

Kaper, J.B., E.F. Remmers, and R.R. Colwell. A medium for the presumptive identification of *Vibrio parahaemolyticus. J. of Food Protect. 43*:936-938, 1980.

Kaper, J.B., E.F. Remmers, H.A. Lockman, and R.R. Colwell. Distribution of *Vibrio parahaemolyticus* in Chesapeake Bay during the summer season. *Estuaries. 4*:322-327, 1981.

Kaper, J.B., H. Lockman, E.F. Remmers, K. Kristensen, and R.R. Colwell. Numerical taxonomy of *Vibrio* spp. isolated from estuarine environments. *Int. J. Syst. Bacteriol. 33*:229-255, 1983.

Kaper, J.B., H. Lockman, M.M. Baldini, and M.M. Levine. A recombinant live oral cholera vaccine. *Biotechnology*. 2:345-349, 1984.

Kaper, J.B., H. Lockman, M.M. Baldini, and M.M. Levine. Recombinant nontoxinogenic *Vibrio cholerae* strains as attenuated cholera vaccine candidates. *Nature*. *308*:655-658, 1984.

Kaper, J.B., H. Lockman, M.M. Baldini, R.E. Black, M.L. Clements, and J.G. Morris. Development and testing of a recombinant live oral cholera vaccine. In *Modern Approaches to Vaccine. Molecular and Chemical Basis of Resistance to Viral, Bacterial, and Parasitic Diseases*. Edited by R. Lerner, and R. Chanock. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1985, in press.

Kaper, J.B., H.A. Lockman, E.F. Remmers, and R.R. Colwell. Numerical taxonomy of *Vibrio* species. *Intl. J. Syst. Bacteriol*. *33*:229-255, 1983.

Kaper, J.B., H.A. Lockman, M.M. Baldini, and M.M. Levine. Development of live oral cholera vaccine candidates through recombinant DNA techniques. In *Advances in Research on Cholera and Related Diarrheas, Vol. 3*. KTK Scientific Publishers, Tokyo, Japan, 1985, in press.

Kaper, J.B., H.B. Bradford, N.C. Roberts, and S. Falkow. Molecular epidemiology of *Vibrio cholerae* resident in the U.S. Gulf Coast. *J. Clin. Microbiol*. *16*:129-134, 1982.

Kaper, J.B., R.K. Campen, R.J. Seidler, M.M. Baldini, and S. Falkow. Cloning of the Kanagawa phenomenon associated hemolysin of *Vibrio parahaemolyticus*. In *Advances in Research on Cholera and Related Diarrheas, Vol. 2*. KTK Scientific Publishers, Tokyo, 229-235, 1983.

Kaper, J.B., R.K. Campen, R.J. Seidler, M.M. Baldini, and S. Falkow. Cloning of the thermostable direct or Kanagawa phenomenon associated hemolysin of *Vibrio parahaemolyticus*. *Infect. Immun*. *45*:290-292, 1984.

Kaper, J.B., S. Moseley, and S. Falkow. Molecular characterization of non-toxigenic *Vibrio cholerae* and related species. *Infect. Immun*. *32*:661-667, 1981.

Karunasagar, I., S.W. Joseph, R.M. Twedt, H. Hada, and R.R. Colwell. Enhancement of *Vibrio parahaemolyticus* virulence by lysed erythrocyte factor and iron. *Infect. Immun*. *46*:141-144, 1984.

Kehoe, M., R. Sellwood, P. Shipley, and G. Dougan. Genetic analysis of K-88 mediated adhesion of enterotoxigenic *E. coli*. *Nature*. *291*:122-126, 1981.

Koopman, J.S., E.A. Eckert, H.B. Greenberg, B.C. Strohm, R.E. Isaacson, and A.S. Monto. Norwalk virus enteric illness acquired by swimming exposure. *Am. J. Epidemiol*. *115*:173-177, 1982.

Koster, F.T., and N.F. Pierce. Parenteral immunization causes antigen-specific cell-mediated suppression of an intestinal IgA response. *J. Immunol*. *131*:115-119, 1983.

Lanata, C.F., J.B. Kaper, R.E. Black, and M.M. Levine. Sensitivity and specificity of the DNA probes for the detection of *E. coli* enterotoxins using the stool blot technique. Submitted.

Lee, C.H., S.L. Moseley, H.W. Moon, S.C. Whipp, C.L. Gyles, and M. So. Characterization of the gene encoding heat-stable toxin II and preliminary molecular epidemiological studies of enterotoxigenic *Escherichia coli* heat-stable toxin II producers. *Infect. Immun*. *42*:264-268, 1983.

Levine, M.M., J.B. Kaper, R.E. Black, and M.L. Clements. New knowledge on the pathogenesis of bacterial enteric infections as applied to vaccine development. *Microbiol. Reviews*. *47*:510-550, 1983.

Levine, M.M., J.P. Nataro, M.M. Baldini, J.B. Kaper, R.E. Black, M.L. Clements, and A.D. O'Brien. The diarrheal response of man to some classical serotype enteropathogenic *Escherichia coli* is dependent on a plasmid encoding an enteroadhesiveness factor. Submitted.

Levine, M.M., P. Ristaino, R.B. Sack, J.B. Kaper, F. Orskov and I. Orskov. Colonization factor antigens I and II and type 1 somatic pili in enterotoxigenic *E. coli*: Relation to enterotoxin type. *Infect. Immun*. *39*:889-897, 1983.

Levine, M.M., R.E. Black, M.L. Clements, and J.B. Kaper. Present status of cholera vaccines. *Biochem. Soc. Trans*. *12*:200-202, 1984.

Levine, M.M., R.E. Black, M.L. Clements, C. Lanata, S. Sears, T. Honda, C.R. Young, and R.A. Finkelstein. Evaluation in humans of attenuated *Vibrio cholerae* El Tor Ogawa Strain Texas Star-SR as a live oral vaccine. *Infect. Immun*. *43*:515-522, 1984.

Levine, M.M., R.E. Black, M.L. Clements, C.R. Young, C. Lanata, S. Sears, T. Honda, and R.A. Finkelstein. Texas Star-SR: Attenuated *Vibrio cholerae* oral vaccine candidate. *Develop. Biol. Standard*. *53*:59-65, 1983.

Levine, M.M., R.E. Black, M.L. Clements, C.R. Young, R.A. Finkelstein, T. Honda, and J.R. Murphy. Clinical and immunological response in man following ingestion of naturally-occurring and laboratory-derived attenuated *Vibrio cholerae*. In *Proceedings of the 16th Joint Conference, U.S.-Japan Cooperative Medical Science Program*: Gifu, Japan, 1980. Edited by S. Kuwahara, and Y. Zinnaka. Fuji Printing Co., Ltd., Tokyo, Japan, 327-334, 1981.

Levine, M.M., R.E. Black, M.L. Clements, C.R. Young, T. Honda, and R.A. Finkelstein. Texas Star-SR: Attenuated *Vibrio cholerae* oral vaccine candidate. *Proceedings of the 17th Joint Conference on Cholera*. U.S.-Japan Cooperative Medical Science Program: October 25-28, Baltimore, Maryland, 1981, in press.

Levine, M.M., R.E. Black, M.L. Clements, D.R. Nalin, L. Cisneros, and R.A. Finkelstein. Volunteer studies in development of vaccines against cholera and enterotoxigenic *Escherichia coli*: A review. In *Acute Enteric Infections in Children: New Prospects for Treatment and Prevention*. Nobel Conference 3. Elsevier/North Holland, Stockholm, 443–459, 1981.

Levine, M.M., R.E. Black, M.L. Clements, L. Cisneros, A. Saah, D.R. Nalin, D.M. Gill, J.P. Craig, C.R. Young, and P. Ristaino. Pathogenicity of non-enterotoxinogenic *Vibrio cholerae* El Tor isolated from sewage water in Brazil. *J. Infect. Dis. 145*:296–299, 1982.

Lockman, H., and J.B. Kaper. Nucleotide sequence analysis of the A2 and B subunits of *Vibrio cholerae* enterotoxin. *J. Biol. Chem. 258*:13722–13726, 1983.

Lockman, H.A., J.E. Galen, and J.B. Kaper. Further analysis of the *Vibrio cholerae* enterotoxin genes: nucleotide sequence analysis of the gene encoding the ADP-ribosylating subunit. *J. Bacteriol. 159*:1086–1089, 1984.

Lockwood, D.E., and D.C. Robertson. Development of a competitive enzyme-linked immunosorbent assay (ELISA) for *Escherichia coli* heat-stable enterotoxin (ST_a). *J. Immunol. Methods.*, 1984, in press.

Lockwood, D.E., A.S. Kreger, and S.H. Richardson. Detection of toxins produced by *Vibrio fluvialis*. *Infect. Immun. 35*:702–708, 1982.

Lockwood, D.E., S.H. Richardson, A.S. Kreger, M. Aiken, and B. McCreedy. *In vitro* and *in vivo* biological activities of *Vibrio fluvialis* and its toxic products. In *Advances in Research on Cholera and Related Diarrheas*. Edited by S. Kuwahara, and N.F. Pierce. KTK Scientific Publishers, Tokyo, Japan, 87–99, 1983.

Marchlewicz, B.A., and R.A. Finkelstein. Immunologic differences among the cholera/coli family of enterotoxins. *Diag. Microbiol. Infect. Dis. 1*:129–138, 1983.

Marchlewicz, B.A., and R.A. Finkelstein. The cholera/coli family of enterotoxins. In *Proceedings of the International Symposium on Bacterial Diarrheal Diseases*, March 23–25, Osaka, Japan, 1982, in press.

McNicol, L.A., J.B. Kaper, H.A. Lockman, E.F. Remmers, W.M. Spira, M.J. Voll, and R.R. Colwell. R-factor carriage in a group F *Vibrio* isolated from Bangladesh. *Antimicrob. Agents Chemother. 17*:512–515, 1980.

McNicol, L.A., S.P. De, J.B. Kaper, P.A. West, and R.R. Colwell. Numerical taxonomy of *Vibrio cholerae* and related species isolated from areas that are endemic and nonendemic for cholera. *J. Clin. Microbiol. 17*:1102–1113, 1983.

McNicol, L.A., S.P. De, J.B. Kaper, P.A. West, and R.R. Colwell. Numerical taxonomy of *Vibrio cholerae* and related species isolated from cholera endemic and non-endemic areas. *J. Clin. Microbiol. 17*:1120–1113, 1981.

McNicol, L.A., T. Barkay, M.J. Voll, and R.R. Colwell. Plasmid carriage in Vibrionaceae and other bacteria isolated from the aquatic environment. *J. Wash. Acad. Sci. 72*:60–66, 1983.

Merson, M.H., R.B. Sack, S. Islam, G. Saklayen, N. Huda, I. Huq, A.W. Zulich, R.H. Yolken, and A.Z. Kapikian. Disease due to enterotoxigenic *Escherichia coli* in Bangladeshi adults: Clinical aspects and a controlled trial of tetracycline. *J. Infect. Dis. 141*:702–711, 1980.

Merson, M.H., R.H. Yolken, R.B. Sack, J.L. Froehlich, H.B. Greenberg, I. Huq, R.W. Black. Detection of *Escherichia coli* enterotoxins in stools. *Infect. Immun. 29*:108–113, 1980.

Monto, A.S., and J.S. Koopman. The Tecumseh Study. XI. Occurrence of acute enteric illness in the community. *Amer. J. Epidemiol. 112*:323–333, 1980.

Monto, A.S., J.S. Koopman, I.M. Longini, and R.E. Isaacson. The Tecumseh study. XII. Enteric agents in the community. *J. Infect. Dis. 148*:284–291, 1983.

Morris, J.G., Jr., J. Michalski, A.C. Wright, J.B. Kaper, J. N. Kaviti, S. Kinoti, A.J. Mohamed, J. Turkish, F.S. Mhalu, and M.M. Levine. Molecular epidemiology of antibiotic-resistant cholera in East Africa. In *Advances in Research on Cholera and Related Diarrheas, Vol. 4*. KTK Scientific Publishers, Tokyo, Japan, 1985, in press.

Moss, J., J.C. Osborne, Jr., P.H. Fishman, S. Nakaya, and D.C. Robertson. *Escherichia coli* heat-labile enterotoxin: Ganglioside specificity and ADP-ribosyltransferase activity. *J. Biol. Chem. 256*:12861–12865, 1981.

Nataro, J.P., M.M. Baldini, J.B. Kaper, R.E. Black, and M.M. Levine. Detection of an adherence factor or enteropathogenic *Escherichia coli* using a DNA probe.Submitted.

Newland, J.W., M.J. Voll, and L.A. McNicol. Serology and plasmid carriage in *Vibrio cholerae*. *Can. J. Microbiol. 30*:1149–1156, 1984.

Nishibuchi, M., and J.B. Kaper. Nucleotide sequence of *Vibrio parahaemolyticus tdh* gene (thermostable direct hemolysin gene). *J. Bacteriol.* 1985, in press.

Nishibuchi, M., J.E. Galen, and J.B. Kaper. The *tdh* gene (thermostable direct hemolysin gene of *Vibrio parahaemolyticus*. In *Advances in Research on Cholera and Related Diarrheas, Vol. 4*. KTK Scientific Publishers, Tokyo, Japan, 1985, in press.

O'Brien, A.D., G.D. LaVeck, D.E. Griffin, and M.R. Thompson. Characterization of *Shigella Dysenteriae* 1 (Shiga) toxin purified by anti-Shiga toxin affinity chromatograhy. *Infect. Immun. 30*:170–179, 1980.

O'Brien, A.D., G.D. LaVeck, M.R. Thompson, and S.B. Formal. Production of *Shigella dysenteriae* 1 (Shiga)-like cytotoxin by *Escherichia coli*. *J. Infect. Dis. 146*:763–769, 1982.

O'Brien, A.D., M.E. Chen, R.K. Holmes, M.M. Levine, and J. Kaper. Environmental and human isolates of *Vibrio cholerae* and *Vibrio parahaemolyticus* produce a *Shigella dysenteriae* 1 (shiga)-like cytotoxin. *Lancet ii*:77–78, 1984.

Orskov, R., R.B. Sack, I. Orskov, and J.L. Froehlich. Changing fecal *Escherichia coli* flora during travel. *Eur. J. Clin. Microbiol. 3*:306–309, 1984.

Owen, R.L., N.F. Pierce, and W.C. Cray, Jr. Effects of bacterial inactivation methods, toxin production and oral immunization on uptake of *Vibrio cholerae* by Peyer's patch lymphoid follicles. In *Advances in Research on Cholera and Related Diarrheas*. Edited by S. Kuwahara, and N.F. Pierce. Martinus Nijhoff, The Hague, in press.

Petschow, B.P., and M.N. Guentzel. The role of motility in adherence of *Vibrio cholerae*. Annual Meeting ASM, abstract, 1985, in press.

Pierce, N.F. Experimental evidence favors oral immunization against cholera. In *Proceedings of the 16th Joint Conference, U.S.-Japan Cooperative Medical Science Program*. Edited by S. Kuwahara, and Y. Zinnaka. Published by Japanese Cholera Panel, U.S.-Japan Cooperative Medical Science Program, Toho University, Tokyo, Japan, 319–326, 1980.

Pierce, N.F. Induction of optimal mucosal antibody responses: Compared effects of age, immunization route(s) and dosing schedule in rats. *Infect. Immun. 43*:341–346, 1984.

Pierce, N.F. Mucosal immunity to cholera. In *Robert Koch Memorial Symposium on Cholera*. Kashiwara, Japan, November 5, 1984, in press.

Pierce, N.F. Suppression of the intestinal immune resonpse to cholera toxin by specific serum antibody. *Infect. Immun. 30*:62–68, 1980.

Pierce, N.F. The experimental basis for oral immunization against cholera. In *Current Topics in Veterinary Medicine and Animal Science, Vol. 12. The Mucosal Immune System*. Edited by F.J. Bourne. Martinus Nijhoff Publishers, The Hague, Netherlands, 461–484, 1981.

Pierce, N.F., and F.T. Koster. Differing migration patterns for IgA memory cells and IgA plasma cells during the enteric immune response to a protein antigen in rats. In *Mucosal Immune System in Health and Disease*. Edited by P.L. Ogra, and J. Bienenstock. 81st Ross Conference on Pediatric Research. Ross Laboratories, Columbus, Ohio, 236–244, 1981.

Pierce, N.F., and F.T. Koster. Priming and suppression of the intestinal immune response to cholera toxoid/toxin by parenteral toxoid in rats. *J. Immunology. 124*:307–311, 1980.

Pierce, N.F., and F.T. Koster. Specific immunologic memory in the secretory mucosal immune system. In *Seminars in Infectious Disease, Vol. IV: Bacterial Vaccines*. Edited by L. Weinstein, B.N. Fields, J.B. Robbins, J.C. Hill, and J.C. Sadoff. Thieme-Stratton, Inc., New York, 19–23, 1982.

Pierce, N.F., and F.T. Koster. Stimulation of intestinal immunity. In *Acute Enteric Infections in Children: New Prospects for Treatment and Prevention*. Edited by T. Holme, J. Holmgren, M.H. Merson, and R. Mollby. Elsevier/North-Holland Biomedical Press, Amsterdam, 413–423, 1981.

Pierce, N.F., and F.T. Koster. The intestinal immune response to cholera toxoid/toxin: Dependence on immunization route and antigen form. In *Cholera and Related Diarrheas: Molecular Aspects of a Global Health Problem*. Nobel Symposium 43, August, Stockholm. Edited by O. Ouchterlony, and J. Holmgren, p.p. 185–194, 1978.

Pierce, N.F., and J.B. Sacci, Jr. Enhanced mucosal priming by cholera toxin and procholeragenoid using a lipoidal amine adjuvant (avridine) delivered in liposomes. *Infect. Immun. 44*:469–473, 1984.

Pierce, N.F., and W.C. Cray, Jr. Cellular dissemination of priming for a mucosal immune response to cholera toxin in rats. *J. Immunology. 127*:2461–2464, 1981.

Pierce, N.F., and W.C. Cray, Jr. Determinants of the localization, magnitude, and duration of a specific mucosal IgA plasma cell response in enterically immunized rats. *J. Immunology. 128*:1311–1315, 1982.

Pierce, N.F., C.R. Alving, E.C. Richardson, and J.B. Sacci, Jr. Enhancement of specific mucosal antibody responses by locally administered adjuvants. In *Proceedings of the 18th Joint Conference, U.S.-Japan Cooperative Medical Science Program*. 1982, in press.

Pierce, N.F., F.T. Koster, and W.S. Barry. Impairment of the enteric secretory immune response by protein-deprivation. In *Nutritional Factors: Modulating Effects on Metabolic Processes*. Edited by R.F. Beers, Jr., and E.G. Bassett. Raven Press, New York, 405–415, 1981.

Pierce, N.F., J.B. Sacci, Jr., and P.F. Engel. Jejunal and mammary gland immune response to enteric immunization in lactating rats. In *Advances in Research in Cholera and Related Diarrheas*. Edited by S. Kuwahara, and N.F. Pierce. Martinus Nijhoff, The Haugue, in press.

Pierce, N.F., J.B. Sacci, Jr., C.R. Alving, and E.C. Richardson. Lipid A enhances mucosal immunogenicity of liposome-associated cholera toxin. *Rev. Infect. Dis. 6*:563–566, 1984.

Pierce, N.F., W.C. Cray, Jr., and J.B. Sacci, Jr. Determinants of the mucosal distribution of specific IgA plasma cells in intestine and trachea. In *Regulation of the Immune Response*. Edited by P.L. Ogra, and D.M. Jacobs. Karger, Basel, 131–140, 1983.

Pierce, N.F., W.C. Cray, Jr., and J.B. Sacci, Jr. Oral immunization of dogs with purified cholera toxin, its B subunit, or a crude culture filtrate of *Vibrio cholerae*: Evidence for synergistic protection by antitoxic and antibacterial mechanisms. *Infect. Immun. 37*:687–694, 1982.

Pierce, N.F., W.C. Cray, Jr., and P.F. Engel. Antitoxic immunity to cholera in dogs immunized orally with cholera toxin. *Infect. Immun. 27*:632-637, 1980.

Pierce, N.F., W.C. Cray, Jr., and P.F. Engel. Antitoxic immunity to cholera in orally immunized dogs. In *Proceedings of the 15th Joint Conference, U.S.-Japan Cooperative Medical Science Program.* p.p. 362-372, 1979

Pierce, N.F., W.C. Cray, Jr., J.B. Kaper, and R.A. Finkelstein. Compared colonizing and immunizing abilities of virulent *Vibrio cholerae* and two avirulent mutants studied in rabbits. In *Advances in Research on Cholerae and Related Diarrheas.* Edited by S. Kuwahara, and N.F. Pierce. Martinus Nijhoff, The Hague, in press.

Pierce, N.F., W.C. Cray, Jr., J.B. Sacci, Jr., and R. Germanier. Oral immunization for cholera: Mucosal antitoxic immunity is important and can probably be safely achieved. In *Advances in Research on Cholera and Related Diarrheas.* Edited by S. Kuwahara, and N.F. Pierce. Martinus Nijhoff, The Hague, Netherlands, 41-48, 1983.

Pierce, N.F., W.C. Cray, Jr., J.B. Sacci, Jr., J.P. Craig, R. Germanier, and E. Furer. Oral immunization against experimental cholera: The role of antigen form and antigen combinations in evoking protection. In *The Secretory Immune System.* Edited by J.R. McGhee, and J. Mestecky. *Ann. N.Y. Acad. Sci. 409*:724-733, 1983.

Pierce, N.F., W.C. Cray, Jr., J.B. Sacci, Jr., J.P. Craig, R. Germanier, and E. Furer. Procholeragenoid: A safe and effective antigen for oral immunization against experimental cholera. *Infect. Immun. 40*:1112-1118, 1983.

Pierce, N.F., W.C. Cray, Jr., J.P. Craig, R. Germanier, and E. Furer. Oral immunization against experimental cholera: The role of antigen form and antigen combinations in evoking protection. *Annals of the New York Academy of Sciences. 409*:724-733, 1983.

Poindexter, N.J., and S.T. Donta. Comparison of the binding of *Vibrio cholerae* and *Escherichia coli* enterotoxins to Y-1 adrenal cells. Program for American Society of Microbiology, Miami Beach, Florida, May, 1982, B117.

Poindexter, N.J., and S.T. Donta. Exposure of cells to cholera toxin alters toxin receptors. American Society of Microbiology, Dallas, Texas, March, 1980.

Rao, M.C., S.A. Orellana, M. Field, D.C. Robertson, and R.A. Giannella. Comparison of the biological actions of three heat-stable enterotoxins: Effects of ion transport and guanylate activity in rabbit ileum. *Infect. Immun. 33*:165-170, 1981.

Remmers, E.F., R.A. Goldsby, and R.R. Colwell. Production and characterization of monoclonal antibodies to cholera toxin. *Infect. Immun. 37*:70-76, 1982.

Remmers, E.F., R.R. Colwell, and R.A. Goldsby. Monoclonal antibodies to cholera toxin. In *Vibrios in the Environment.* Edited by R.R. Colwell, J. Wiley and Sons, New York, 161-172, 1983.

Resnick, I.G., C.W. Ford, G.M. Shackleford, and L.J. Berry. Improved protection against cholera in adult rabbits with a combined flagellar-toxoid vaccine. *Infect. Immun. 30*:375-380, 1980.

Richardson, S.H., and R.E. Kuhn. H-2 modulation of cholera enterotoxin effects in SAM (Sealed Adult Mice). In *Proceedings of the 19th Joint Conference on Cholera of the U.S.-Japan Cooperative Medical Science Program*, in press.

Richardson, S.H., J.C. Giles, and K.S. Kruger. Sealed adult mice: New model for enterotoxin evaluation. *Infect. Immun. 43*:482-486, 1984.

Roberts, N.C., R.J. Siebling, J.B. Kaper, and H.B. Bradford. Vibrios in the Louisiana Gulf Coast environment. *Microbiol. Ecology. 8*:299-312, 1982.

Robertson, D.C., L.A. Dreyfus, and J.C. Frantz. Chemical and immunological properties of *Escherichia coli* heat-stable enterotoxin. *Progress in Food and Nutrition-Science, 7*:147-156, 1983.

Robins-Browne, R.M., C.R. Young, M.M. Levine, and J.P. Craig. Microtiter enzyme-linked immunosorbent assay for immunoglobulin G cholera antitoxin in humans: Sensitivity and specificity. *Infect. Immun. 27*:497-500, 1980.

Ryer, R.W., C.A. Oquist, H. Greenberg, D.N. Taylor, F. Orskov, I. Orskov, A.Z. Kapikian, and R.B. Sack. Travelers' diarrhea in Panamanian tourists in Mexico. *J. Infect. Dis. 144*:442-448, 1981.

Ryder, R.W., H. Greenberg, N. Singh, G. Oro, A. deGuardia, R.B. Sack, and A.Z. Kapikian. Seroepidemiology of heat-labile enterotoxigenic *Escherichia coli* and Norwalk virus infections in Panamanians, Canal Zone residents, Apache Indians, and United States Peace Corps Volunteers. *Infect. Immun. 376*:903-906, 1982.

Sack, R.B. Antimicrobial prophylaxis of travellers' diarrhoea: A summary of studies using doxycycline or trimethoprim and sulphamethoxazole. *Scand. J. Gastroenterology 18*:111-117, 1982.

Sack, R.B. Enterotoxigenic *Escherichia coli*: Identification and characterization. *J. Infect. Dis. 142*:279-286, 1980.

Sack, R.B. Enterotoxigenic *Escherichia coli* mediated diarrheal disease in humans. (Enfermedad diarrheica causada por *Escherichia coli* enterotoxigenica en humanos.) *Diagnostico. 11(92)*:66-72, 1983.

Sack, R.B. *Escherichia coli* and acute diarrheal disease. *Ann. Int. Med. 94*:129-130, 1981.

Sack, R.B. Pathogenesis and pathophysiology of diarrheal diseases caused by *Vibrio cholerae* and enterotoxigenic

Escherichia coli. In *Cholera and Related Diarrheas, 43rd Nobel Symposium*, Stockholm, 1978, Karger, Basel, 53–63, 1980.

Sack, R.B., and J.L. Froehlich. Berberine inhibits intestinal secretory response of *Vibrio cholerae* and *Escherichia coli* enterotoxin. *Infect. Immun. 35*:471–475, 1982.

Sack, R.B., M. Santosham, J.L. Froehlich, C. Medina, F. Orskov, and I. Orskov. Doxycycline prophylaxis of travelers' diarrhea in Honduras, an area where resistance to doxycycline is common among enterotoxigenic *Escherichia coli*. *Am. J. Trop. Med. Hyg. 33*:460–466, 1984.

Santosham, M., B. Burns, V. Nadkarni, C. O'Donovan, S.T. Foster, S. Garrett, R. Pathak, and R.B. Sack. Oral rehydration therapy for acute diarrhea in ambulatory U.S. children: A double-blind comparison of four different solutions. *Pediatrics*, in press.

Santosham, M., E. Carrera, and R.B. Sack. Oral rehydration therapy in well-nourished ambulatory children. *Am. J. Trop. Med. Hyg. 32*:804–808, 1983.

Santosham, M., R.B. Sack, E. Lochlear, S. Foster, S. Garret, D. Rousey, S. Evans, and R. Black. Storing oral rehydration solution. *Lancet. i*:797, 1982.

Santosham, M., R.B. Sack, J. Froehlich, H. Greenberg, R. Yolken, A. Kapikian, C. Javier, C. Medina, F. Orskov, and I. Orskov. Biweekly prophylactic doxcycycline for travelers' diarrhea. *J. Infect. Dis. 143*:598–602, 1981.

Santosham, M., R.B. Sack, J.L. Froehlich, L. Aurelian, H.B. Greenberg, R. Yolken, A.Z. Kapikian, C. Javier, C. Medina, F. Orskov, and I. Orskov. Bioweekly prophylactic doxcycycline for travelers' diarrhea. *Current Chemotherapy and Infectious Disease Proceedings of the 11th ICC and the 19th ICAAC American Society of Microbiology*, 922–924, 1980.

Santosham, M., R.S. Daum, L. Dillman, J.L. Rodriguez, S. Luque, R. Russell, M. Kourany, R.W. Ryder, A.V. Bartlett, A. Rosenberg, A.S. Benenson, and R. B. Sack. Oral rehydration therapy of infantile diarrhea. A controlled study of well-nourished children hospitalized in the United States and Panama. *N. Engl. J. Med. 306*:1070–1076, 1982.

Santosham, M., S. Foster, R. Reid, R. Bertrando, R. Yolken, B. Burns, and R.B. Sack. Role of soy-based, lactose-free formula during the treatment of acute diarrhea. *Pediatrics*, in press.

Sciortino, C.V., and R.A. Finkelstein. *Vibrio cholerae* express iron-regulated outer membrane proteins *in vivo*. *Infect. Immun. 42*:990–996, 1983.

Sciortino, C.V., and R.A. Finkelstein. *Vibrio cholerae* iron-related outer membrane proteins *in vivo*. In *Advances in Research on Cholera and Related Diarrheas*. KTK Scientific Publishers, Tokyo, Japan, 1984, in press.

Shandera, W.X., B. Hafkin, D.L. Martin, J.P. Taylor, D.L. Maserang, J.G. Wells, M. Kelly, K. Ghandi, J.B. Kaper, J.V. Lee, and P.A. Blake. Persistence of cholera in the United States. *Am. J. Trop. Med. Hyg. 32*:812–817, 1983.

Sheridan, J.F., L. Aurelian, G. Barbour, M. Santosham, R.B. Sack, and R.W. Ryder. Traveler's diarrhea associated with rotavirus infection: Analysis of virus-specific immunoglobulin classes. *Infect. Immun. 31*:419–429, 1981.

Shipley, P.L., A.D. Allen, and T.N. Swanson. Cointegrate formation between plasmids carrying virulence factor and antibiotic resistance genes in *E. coli*. *FEMS Microbiol. Lett. 20*:365–368, 1983.

Shipley, P.L., G. Dougan, and S. Falkow. Identification and cloning of the genetic determinant that encodes for the K88 adherence antigen. *J. Bacteriol. 145*:920–925, 1981.

Sigel, S.P., R.A. Finkelstein, and C.D. Parker. Ability of an avirulent mutant of *Vibrio cholerae* to colonize in infant mouse upper bowel. *Infect. Immun. 32*:474–479, 1981.

Singh, A., M.W. Lechevallier, and G.A. McFeters. Reduction in mouse virulence by *Yersinia enterocolitica* after exposure to sublethal concentration of copper. To be presented at the National Meeting of the American Society for Microbiology, March, 1985.

Singleton, F.L., R.W. Attwell, M.S. Jangi, and R.R. Colwell. Influence of salinity and organic nutrient concentration on survival and growth of *Vibrio cholerae* in aquatic microcosms. *Appl. Environ. Microbiol. 43*:1080–1085, 1982.

Spira, W.M., and R.B. Sack. Kinetics of early cholera infection in the removable intestinal tie-adult rabbit diarrhea model. *Infect. Immun. 35*:952–957, 1982.

Spira, W.M., R.B. Sack, and J.L. Froehlich. Development of a simple adult rabbit model for cholera and ETEC infection. In *Proceedings of the 16th Joint Conference, U.S.-Japan Cooperative Medical Science Program*, 1980.

Spira, W.M., R.B. Sack, and J.L. Froehlich. Simple adult rabbit model for *Vibrio cholerae* and enterotoxigenic *Escherichia coli* diarrhea. *Infect. Immun. 32*:739–747, 1981.

Sugarman, B., and S.T. Donta. Factors affecting the adherence of *Enterobacteriaceae* to mammalian cells. *Proceedings of the 15th Joint Conference on Cholera*. NIH publication #80, 2003, 84–93, 1980.

Snyder, J.D., D.T. Allegra, M.M. Levine, J.[illegible] Craig, J.C. Feeley, W.F. DeWitt, and P.A. Blake. Serological studies of naturally acquired *Vibrio cholerae* O group 1 infection in the United States. *J. Infect. Dis. 143*:182–187, 1981.

Thompson, M., H. Brandwein, M. Labine-Racke, and R. Giannella. Simple and reliable enzyme-linked immunosorbant assay with monoclonal antibodies for detection of *Escherichia coli* heat-stable enterotoxin. *J. Clin. Microbiol. 20*:59–64, 1984.

Thompson, M.R., H. Brandwein, M. LaBine-Racke, and R.A. Gianella. Simple and reliable ELISA assay with monoclonal antibodies for detection of *Escherichia coli* heat-stable enterotoxin. *J. Clin. Microbiol. 20*:59–64, 1984.

Thompson, M.R., R.A. Giannella, A. Deutsch, and H. Brandwein. Monoclonal antibodies directed against *E. coli* heat-stable enterotoxins (STa). In *Proceedings of the 19th Joint U.S.-Japan Conference on Cholera*. October, 1983, in press.

Thorne, G.M., C.F. Deneke, and S.L. Gorbach. Pili, hemagglutination, and adherence. In *Attachment of Organisms to the Gut Mucosa, Vol. I.* Edited by E.C. Boedeker. CRC Press, Florida. 79-90, 1984.

Thorne, G.M. , S.W. Rothman, and S.L. Gorbach. Novel *Escherichia coli* causing diarrhea, bacteremia and fever in a human. *J. Innfect. Dis.*, 1985, in press.

Tokunaga, E., W.C. Cray, Jr., and N.F. Pierce. Colonization and immunization of adult rabbits by oral inoculation with virulent and avirulent *Vibrio cholerae*. In *Proceedings of the 18th Joint Conference, U.S.-Japan Cooperative Medical Science Program*. 1982, in press.

Tokunaga, E., W.C. Cray, Jr., and N.F. Pierce. Compared colonizing and immunizing efficiency of toxinogenic (A^+B^+)*Vibrio cholerae* and an A^-B^+ mutant (Texas Star-SR) studied in adult rabbits. *Infect. Immun. 44*:364-369, 1984.

Trevino, L., M. Beckius, and M.N. Guentzel. Interactions of *Vibrio cholerae* with intestinal mucin. MBRS Symposium. *204*:abstract, 1983.

Viscidi, R., B. Laughon, M. Hanvanich, R. Yolken, and J.G. Bartlett. Improved enzyme immunoassays for the detection of antigens in fecal specimens. *J. Immunol. Methods. 67*:129-143, 1984.

Wall, V.W., A.S. Kreger, and S.H. Richardson. Production and partial characterization of a *Vibrio fluvialis* cytotoxin. *Infect. Immun. 46*:773-777, 1984.

West, P.A., and R.R. Colwell. Identification and classification of *Vibrionaceae*—An overview. In *Vibrios in the Environment*. Edited by R.R. Colwell. J. Wiley and Sons, New York, 285-362, 1983.

Wolfe, K.H., H. Brandwein, A. Deutsch, and D. Reed. A diagnostic kit for the detection of the K99 *E. coli* pilus in feces. Presented at the Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians, Fort Worth, Texas, October, 1984.

Wright, W., C. Wysowski, and M.N. Guentzel. Virulence determinants of *Vibrio cholerae:* Selected enzyme activities and hemagglutinin. MBRS Symposium. *10(37)*:abstract, 1984.

Xu, H.-S., N.C. Roberts, F.L. Singleton, R.W. Attwell, D.J. Grimes, and R.R.Colwell. Survival and viability of nonculturable *Escherichia coli* and *Vibrio cholerae* in the estuarine and marine environment. *Microb. Ecol. 8*:313-323, 1982.

Xu, H.-S., N.C. Roberts, L.B. Adams, P.A. West, R.J. Siebeling, A. Huq, M.I. Huq, R. Rahman, and R.R. Colwell. An indirect fluorescent antibody staining procedure for detection of *Vibrio cholerae* serovar 01 cells in aquatic environmental samples. *J. Microb. Methods. 2*:221-231, 1983.

Young, C.R., M.M. Levine, J.P. Craig, and R. Robins-Browne. Microtiter enzyme-liked immunosorbent assay for immunoglobulin G cholera antitoxin in humans: Method and correlation with rabbit skin vascular permeability factor technique. *Infect. Immun. 27*:492-496, 1980.

Joint Scientific Conferences

CHOLERA PANELS

Date: October 6-8, 1980

Place: Gifu, Japan

Summary: Forty-two papers were presented in the presence of 81 participants. As usual, epidemiology and pathology of cholera and related diarrheal diseases, bacteriology and genetics of the causative organisms, and immunology and development of vaccines were the main subjects. Special attention was paid to the topics such as the regulatory mechanism in the toxin production by *V. cholerae*, the mode of binding to the cell surface and subsequent internalization of cholera toxin into the cell, and the genetic analysis of the plasmids of enterotoxigenic *E. coli*. Results of the volunteer study of peroral live cholera vaccine were also presented. Some important findings suggesting the possible participation of factors other than cholera enterotoxin in diarrhea were reported, and it was thought that further investigation would be necessary prior to practical application of such a live vaccine. Another important topic was an outbreak of cholera caused by a multiply antibiotic-resistant *V. cholerae*.

Date: October 25-28, 1981

Place: Hyatt Regency Hotel, Baltimore, Maryland

Summary: This important meeting was attended by more than 110 individuals, including five members of the Japanese Cholera Panel and nine other foreign attendees. Forty papers were chosen for presentation from among 60 submitted abstracts. The program covered the following major topics: immunology and vaccine development, mucosal immunology, bacteriology and virulence factors, clinical studies, genetics, and enterotoxins.

Investigators at the Johns Hopkins University reported on their studies in animals to show that oral administration of procholeragenoid is an effective mucosal immunogen. Other investigations by this group support the notion that mucosal immune response to cholera toxin and other antigens of *V. cholerae* act synergistically to prevent experimental cholera. In the future, a multivalent oral vaccine containing procholeragenoid rather than active toxin may prove both safe and protective in man.

Cholera lectin has been isolated to homogeneity by investigators at the University of Missouri. Preliminary studies suggest that attachment by cholera vibrios to intestinal epithelium may involve a consortium of factors in which cholera lectin plays a major role.

In a prospective controlled clinical trial in Mexico, investigators at the University of Texas Medical School demonstrated Bicozamycin to be efficacious and safe in the treatment of acute travelers' diarrhea mediated by toxigenic *E. coli*.

Other outstanding papers were presented by both U.S. and Japanese investigators in the areas of toxinology, genetics, and bacteriology.

The Japanese Cholera Panel agreed to publish the proceedings of this important conference.

Date: November 28-December 1, 1982

Place: Kirashiki, Okayama, Japan

Summary: Several important observations were presented on enterotoxins. Among them were: confirmation of antigenic differences between heat-labile toxins (LT's) of *E. coli* of human and porcine origin; determination of base sequences of LT gene on Ent plasmid; and determination of primary structure as well as artificial synthesis of heat-stable toxin of *E. coli* (ST). It was also reported that gene analysis had been introduced into the study of virulence factors, and into the study of the causative organisms of cholera-like disease, including enterotoxigenic *E. coli*, *V. parahaemolyticus* and others. Basic studies for the development of effective vaccines were described by many investigators.

Date: October 17-19, 1983

Place: National Institutes of Health, Bethesda, Maryland

Summary: Over 130 individuals attended this conference; including five members of the Japanese Cholera Panel and 10 additional Japanese guests. Thirty-nine papers were chosen for presentation from among 62 submitted abstracts. The program consisted of the following sections: epidemiology, bacteriology and virulence factors, toxinology, genetics, immunology and vaccine development, and pathophysiology.

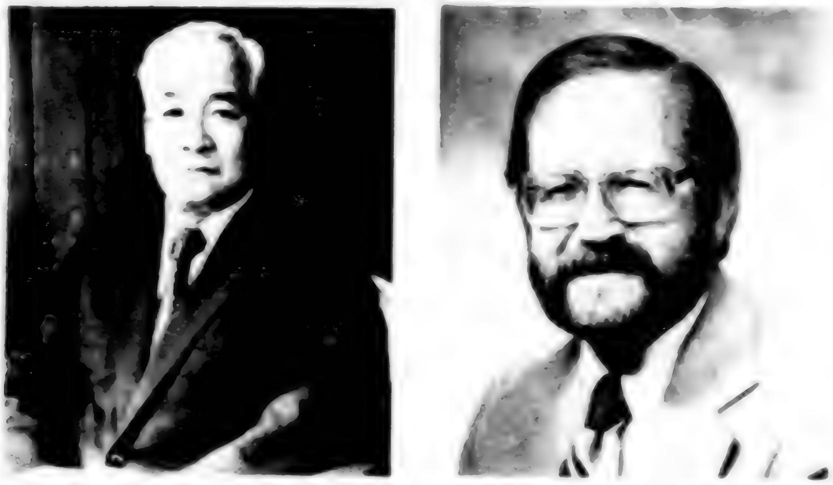
The most important development in cholera research reported at the meeting dealt with the discovery and early characterization of hitherto undetected enterotoxin(s) (*E. coli* heat-stable toxins and Shiga-like toxin) by a number of investigators working with *V. cholerae* and *E. coli*. Their discovery will have great impact on basic research directed at improved vaccine development. Several live, oral cholera vaccine candidate strains developed using recombinant DNA techniques were reported by investigators affiliated with the University of Maryland and Harvard University. The scientific papers presented by the Japanese and American scientists were of the highest quality. The proceedings of this conference were scheduled to be published by the Japanese Cholera Panel.

Date: November 6–8, 1984

Place: Nara, Japan

Summary: At this joint conference, cloning of the hemolysin gene of *V. cholerae* as well as of *V. parahaemolyticus*, isolation and purification of ST receptor on the intestinal mucosa, and the mechanism of biosynthesis and release of cholera enterotoxin were major subjects. From the comparative studies on the base sequence of the genes of cholera and related enterotoxins, the evolutionary relationship of these toxins was discussed. The biological activity of LT was analyzed based on its amino acid sequence. To develop a more effective vaccine, the immunologic studies on cholera have been continued. For instance, the role of M cells in Peyer's patches on antigen recognition and subsequent transfer to lymphocytes was analyzed. Since this year was the centennial of Robert Koch's publication on the pathogenic importance of *V. cholerae*, a very important symposium commemorating R. Koch was held a day before the main conference, and cholera studies during the past 100 years were summarized. Attendees came from many parts of the world.

ENVIRONMENTAL MUTAGENESIS AND CARCINOGENESIS PANEL



Drs. Takeo Suzuki and Frederick J. de Serres, Chairmen respectively of the Environmental Mutagenesis and Carcinogenesis Panel, U.S.-Japan Cooperative Medical Science Program

Dr. Takeo Suzuki, Chairman (1981– ; Member 1972–81)
Director General
Institute of Public Health
Shirokanedai 4-6-1, Minato-ku
Tokyo 108, Japan

Dr. Frederick J. de Serres, Chairman (1972–)
Associate Director
Office of the Associate Director for Genetics
National Institutes of Health
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, North Carolina 27709

PANEL MEMBERS

Japan

Dr. Taijiro Matsushima (1977–)
Professor
Department of Molecular Oncology
Institute of Medical Science
Tokyo University
4-6-1, Shirokanedai, Minato-ku
Tokyo 108, Japan

Dr. Takashi Sugimura (1972–)
President
National Cancer Center
5-1-1, Tsukiji, Chuo-ku
Tokyo 104, Japan

Dr. Yataro Tazima (1972–)
Director
Institute of Silk Worm Genetics and Breeding
1054, Iikura, Ami-cho
Inashiki-gun, Ibaragi Prefecture
Tokyo 300-04, Japan

United States

Dr. Mortimer Mendelsohn (1979–)
Associate Director for Biomedical and
Environmental Research Program
Biomedical Sciences Division L-452
Lawrence Livermore Laboratory
P.O. Box 5507
Livermore, California 94550

Dr. John J. Mulvihill (1979–)
Head, Clinical Genetics Section
Clinical Epidemiology Branch
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20205

Dr. Irving Selikoff (1979–)
Professor of Community Medicine
Department of Community Medicine
Mt. Sinai School of Medicine
City University of New York
New York, New York 10029

Dr. Shozo Takayama (1982-)
Director
National Cancer Center
Research Institute
5-1-1, Tsukiji, Chuo-ku
Tokyo 104, Japan

Former Members

Dr. Tadashi Yamamoto, Chairman
(1972-1981) Deceased
Director
Tokyo Metropolitan Institute of Medical Science
Honkomagome 3-18-22, Bunkyo-ku
Tokyo 113, Japan

Japanese Environmental Mutagenesis and Carcinogenesis Panel

BROAD GOALS

The primary research activities have progressed according to the panel's guidelines which were revised in 1978–79. These guidelines concentrate on studies to determine the effects of chemical compounds on human health, especially mutagens and carcinogens, which have been introduced into the environment by man.

A. Detection of mutagens and carcinogens.

1. Development, refinement, and validation of mutagenicity assay systems for use in mass screening programs;
2. Improvement in the ability to make human risk estimations;
3. Investigation of factors which modify mutagenic and carcinogenic activities;
4. Investigation of structure-activity relationships of both mutagens and carcinogens;
5. Detection of mutagens, carcinogens, and tumor promoters in the environment;
6. Detection of mutagens and carcinogens in foods.

B. Surveillance to determine the occurrence and effects of mutagens and carcinogens in the environment on humans.

1. Development of methodology for monitoring human populations for mutations;
2. Application of human monitoring methods, surveillance of high-risk populations to genetic damage and carcinogenesis;
3. Development of methodologies for monitoring the environment for the presence of mutagens and carcinogens;
4. Development of methodologies for epidemiological investigations of human populations suspected or known to have been exposed to mutagens or carcinogens.

PROGRESS IN PRIORITY AREAS

Development, Refinement, and Validation of Short-Term Test Systems for Mutagens and Carcinogens

Several members of the panel participated in the international program for the evaluation of mutagenicity test systems which were widely used as primary screening methods of potential carcinogenic compounds. The Salmonella mutation test, the *E. coli* mutation test, and the Rec-assay were studied on 42 coded chemical compounds. The test systems were validated by comparison with other systems and test data.

In the test of histidine containing samples such as foods by the Ames' Salmonella mutation test, histidine should be eliminated from the sample before the test. New tester strains, streptomycin-dependent Salmonella TA98 and TA100, were isolated for use in new bacterial mutation test systems which were not affected by histidine in test samples.

A new cell line, CHL/IU was isolated and established from Chinese hamster fibroblasts. This cell line has a high sensitivity to mutagens and appears suitable for the *in vitro* chromosome aberration test. Many environmental chemical compounds were tested by this new chromosome aberration test.

Sister chromatid exchange was studied using several culture cell lines and a suitable cell line was established. Incorporation of an *in vitro* metabolic activator into the sister chromatid exchange assay was also studied, and the method was developed and established.

A new *in vitro* mutation assay using cultured hamster cells with diphtheria-toxin resistance as a phenotypic selection marker was developed. This method could be used as an *in vivo* mutation assay for the detection of somatic mutation in laboratory animals and humans.

An *in vitro* unscheduled DNA synthesis method detecting the repair synthesis of damaged DNA caused by chemical substances was studied using the organ cultures of several tissues of human origin.

Using the organ specificity of carcinogens, *in vivo/in vitro* short-term assay detecting stomach carcinogens was developed. Chemical compounds were administered to rats by a stomach tube and then the induction of unscheduled DNA synthesis, total DNA synthesis, and ornithine decarboxylase were measured, after *in vitro* incubation of mucosa from the glandular stomach. By this assay method, both tumor initiators and tumor promoters of stomach carcinogenesis could be detected.

Investigation of Factors Which Modify Mutagenic and Carcinogenic Activities

The occurrence of anti-mutagens, which block mutation induction processes by chemical compounds, and desmutagens, which destroy biological activities of mutagenic compounds, was studied in several pure and crude compounds. A desmutagen was found in the juice of several fresh vegetables and fruits. An active principle was isolated and identified as a protein having peroxidase-like activity.

Mutagenic activities of heterocyclic amine-type mutagens isolated from pyrolysis products of amino acids were destroyed by the action of peroxidases plus H_2O_2 and also by treatment of HNO_2.

Investigation of Structure-Activity Relationships of Both Mutagens and Carcinogens

The mutagenicity of naturally occurring mutagens, flavonoids, anthraquinones, naphthoquinones, and benzoquinones, present in edible plants such as vegetables and fruits, and in medicinal plants, were studied for the possible relationship between chemical structure and mutagenic activity. Mutagenic activities were manifested by introduction of hydroxyl groups into these compounds. The position in the compounds and the types and numbers of hydroxyl groups modified the mutagenic activities.

Because of the large human intake of flavonoids, which are widely present in edible vegetables and fruits, risks of these compounds on human health should be clarified. Quercetin, which is widely distributed in plants, was selected as an example of a flavonoid. The carcinogenicity of quercetin was studied using rats, mice, and hamsters. Although quercetin was mutagenic in several *in vitro* mutation tests, it was not carcinogenic in small rodents.

Detection of Mutagens, Carcinogens, and Tumor Promoters in the Environment

Nitropyrenes were identified as one cause of the mutagenic activity detected in diesel engine exhausts and atmosphere air-pollutants. These compounds are very potent mutagens. Dinitropyrenes were shown to be carcinogenic in animal bioassays, but the carcinogenicity of mononitropyrenes was not detected.

An active principle of the carcinogenic bracken fern was isolated and identified as ptaquiloside, a norsesquiterpene glucoside. Its carcinogenicity and toxicity were detected in rats and calves. This is consistent with the effects of bracken fern on these animal species.

New classes of tumor promoters with chemical structures different from known tumor promoters were discovered in the culture media of Streptomyces and in blue green algae seaweed. These were the indole alkaloids, teleocidin and dihydroteleociden from Streptomyces, and lyngbyatoxin from seaweed, and the polyacetates, aplysiatoxin and debromoaplysiatoxin, from seaweed.

Detection of Mutagens and Carcinogens in Food

The pyrolysis mutagens, Trp-P-1 and Trp-P-2 isolated from pyrolysates of tryptophan, Glu-P-1 and Glu-P-2 isolated from pyrolysates of glutamic acid; and AalphaC and MeAalphaC isolated from pyrolysates of protein were shown to be carcinogens in animal bioassay tests.

New potent mutagens having unique chemical structures were isolated and identified as IQ and MeIQ from broiled sun-dried sardines, and MeIQx and IQ from broiled beef and heated beef extracts. These new classes of mutagens have an amino-imidazole skeleton as a common chemical structure. The carcinogenicity of IQ was also shown by animal bioassay.

Glu-P-1, Glu-P-2, and IQ induced multiple tumors in several organs such as liver, small intestine, large intestine, Zymbal gland, and brain of rats.

The precursors of amino-imidazole type mutagens, MeIQx, IQ, and MeIQ were studied. MeIQx was isolated from mixtures of creatinine, amino acids, and glucose which were heated to about 130°C. The amino-imidazole type mutagens were probably produced in meats through the Maillard reaction.

New mutagens, 7,8-DiMeIQx and 4,8-DiMeIQx, were isolated from the heated mixture of creatinine, glycine, and glucose and of creatinine, alanine, and glucose, respectively.

1-Methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid and tyramine were isolated and identified as precursors of mutagens which were produced by a treatment of soy sauce with nitrite. The former was produced from tryptophan, and the latter was produced from tyrosine during the fermentation process of the soy sauce. These precursors of mutagens in soy sauce may produce mutagens in the stomach by the reaction with nitrite. The carcinogenicity of co-administration of soy sauce and nitrite is being studied.

Developmental Methodology of Monitoring Human Populations for Mutations

Chromosome aberrations and sister chromatid exchanges were studied in the PHA-stimulated lymphocytes obtained from peripheral human blood to determine if they could be used to monitor human exposure to environmental chemicals.

Retinoblastomas and Wilms' tumors (nephroblastomas) were identified as markers in human population for mutations in germ cells and somatic cells. Ninety-five percent of cases of retinoblastomas occur sporadically. Sporadic bilateral retinoblastomas were usually due to dominant germinal mutations, and most cases of sporadic unilateral retinoblastomas were due to nonhereditary somatic mutations. Sporadic retinoblastomas may be used for surveillance of both germinal and somatic mutations.

Application of Human Monitoring Methods, Surveillance of High-Risk Populations to Genetic Damage and Carcinogenesis

The frequencies of chromosome aberration and of sister chromatid exchange in PHA-stimulated lymphocytes were increased in workers engaged in stainless steel welding. This was due to the exposure to particles containing chromium and to ozone. The frequency of sister chromatid exchange in smoking workers was significantly higher than that in nonsmoking workers. The frequency of sister chromatid exchange was elevated in PHA-stimulated lymphocytes which were obtained from the peripheral blood of workers who sprayed dyes to detect metal cracks. Sister chromatid exchange was shown to be a good marker for monitoring human exposure to environmental mutagens.

Inducibility of sister chromatid exchange in peripheral blood lymphocytes by known mutagens and clastogens appeared to show individual variability, but some people showed high inducibility of sister chromatid exchange by mutagens. This inducibility of sister chromatid exchange could possibly be used for detecting high-risk groups exposed to environmental chemicals.

SUMMARY OF 13 YEARS OF PROGRESS AND ITS IMPACT

Availability of short-term mutagenicity tests, especially the Salmonella mutation test, the Rec-assay, the silk-worm mutagenicity test, and the *in vitro* chromosome aberration test were developed, studied, and improved. These tests now are widely used for detecting environmental mutagens and carcinogens. The relationship between mutagenicity and carcinogenicity of chemicals was clarified, and it was proved that all *in vitro* mutagens were not necessarily carcinogenic. Short-term *in vitro* mutagenicity tests were widely used as primary screening tests for potential carcinogens. Several scientists on this panel in close cooperation with members of the U.S. panel were actively involved in the WHO–IPCS international program for the evaluation of mutagenicity tests as short-term tests for potential carcinogens.

This panel was established in 1972, the same year as the Environmental Mutagen Society of Japan was established, following the American Environmental Mutagen Society and the European Environmental Mutagen Society. In the same year, the mutagenicity of AF-2, a major food additive in use then, was discovered by the *in vitro* chromosome aberration test using human lymphocytes. Public interest on the mutagenicity of environmental chemicals began to increase. The first workshop of this panel was held in July of 1974 and considered the problems of AF-2. The genetic toxicity and carcinogenicity of AF-2 was discussed because of the possible relationship between mutagenicity and carcinogenicity. The carcinogenicity of AF-2 was shown by the induction of forestomach tumors in mice. The usage of AF-2 was banned soon after this workshop. Based on this experience and the worldwide increasing usage and interest in environmental mutagens, several mutagenicity tests, such as Salmonella and *E. coli* bacterial mutation tests and *in vitro* chromosome mutation tests were adopted as a battery of toxicity tests for the safety evaluation of medical drugs, medicinal preparations, cosmetics, food-additivies, feed-additives, pesticides, and herbicides. The bacterial mutagenicity test data were also included in the notification of new chemical usage prepared for new industrial processes. Such lists could aid in the prevention of occupational cancer after 1979.

The formation of mutagens during the cooking of foods was first discovered in 1976 by Japanese studies using the Salmonella mutagenicity test. More than 10 new mutagens were isolated from cooked foods and pyrolysates of amino acids and proteins. Their carcinogenicity also has been shown by bioassays in mice and rats. This new finding triggered worldwide interest in this new research area. Workshops on this topic were organized by this panel in 1981 and again in 1984, and cooperative research studies between Japan and U.S. investigators were initiated. Studies on the significance and risk estimation of mutagens and carcinogens in foods, especially cooked foods, for the causes of human cancer have been started.

FUTURE GOALS

1. *In vitro* short-term assay tests to be used as primary screens for potential carcinogens were established. It became clear that all mutagens detected by *in vitro* short-term assay were not necessarily *in vivo* genetic toxicants or carcinogens. It is urgent and important that appropriate *in vivo* short-term tests be developed for the evaluation of the effects on laboratory animals and humans by mutagens—potential carcinogens, which have been detected in the environment by these *in vitro* short-term mutation tests.
2. Although the mutagenicity tests are effective short-term assays for detecting tumor initiators, the detection of tumor promoters simultaneously with tumor initiators is important for the purpose of cancer prevention. Development of the short-term assay for tumor promoters is also an urgent and important subject.
3. Development of methods for monitoring mutations in human populations and of automated systems for detecting chromosome aberration, sister chromatid exchange, and unscheduled DNA synthesis are important goals, as in surveillance of high-risk populations genetically predisposed to genetic damage and carcinogenesis.
4. The study of oncogene activation in laboratory animals and humans by environmental mutagens and carcinogens, and its application for monitoring high-risk subpopulations are also important future goals.

U.S. Environmental Mutagenesis and Carcinogenesis Panel

BROAD GOALS

Human exposure to toxic chemicals—occupationally, through synthetic chemicals found in food, water, or air, or as naturally occurring chemicals in the environment—can result in diverse health effects ranging from nondetectable to lethal. Effects may be expressed immediately or decades following initial exposure. The two environmentally related health effects of concern to this panel are cancer and heritable genetic disorders. They have two characteristics in common. The effects are not generally associated in time with exposure to the causal agent, i.e., the effect is not immediate. The health effects are largely irreversible. In each case, the panel's goal is to promote preventive health measures through the identification of mutagens and carcinogens, and, subsequently, to eliminate or restrict human exposures to such chemicals.

Development of better methods to screen chemicals before they are released into the environment and to provide thorough evaluation of chemicals already present in the environment is the primary activity area of this panel. Exploratory experiments on various classes of chemicals (drugs, food additives, pesticides, cosmetics, industrial compounds, etc.) showed numerous examples of agents with genetic activity in experimental organisms. The problem is to determine their effect on humans.

Another panel goal is to develop test methods for human population monitoring to detect genetic damage that may lead to adverse human health effects related to genetic disease and cancer. To ensure validity of any screening program, new approaches need to be developed for population monitoring and epidemiology. Better tests are required to evaluate the effects of accidental or deliberate exposure to mutagenic agents on human somatic cells and germ cells. Exposure of germ cells may induce damage transmissable to future generations. Some effects are recessive and not expressed for many generations. Dominant gene mutations and various chromosome aberrations have immediate expression and may produce abortion or various birth defects in a viable child. Simple tests need development for use in evaluating readily available samples of body tissues of workers in various industries which use highly toxic reagents.

The joint panel activities are characterized by a high level of interaction and frequent communication between U.S. and Japanese participants. The problems identified by the joint panels during the past 5 years were acted upon by a variety of mechanisms within the context of the U.S.-Japan Cooperative Medical Sciences Program and through our contact with other international organizations. The Joint Panel Conferences and Workshops will provide a forum for problem identification and resolution in this important research area during the next 5-year period.

PROGRESS IN PRIORITY AREAS

Summary of the Third Five Years (1980–85)

A review of the guidelines adopted in 1978 shows that the activities of the U.S. Environmental Panel focused on the detection of chemical pollutants with mutagenic and carcinogenic activity with regard to (1) development, refinement, and validation of mutagenicity assay systems for use in mass-screening programs; (2) support of investigations which improve our ability to make human-risk estimations; (3) support investigations of factors which modify mutagenic activity; and (4) support investigations of structure-activity relationships of both mutagens and carcinogens. Another area of activity was surveillance to determine the occurrence and effects of mutagens and carcinogens in the environment by (1) development of methodology for monitoring human populations for mutations in somatic cells and germ cells, (2) application of human monitoring methods to determine baseline mutation frequencies, (3) development of methodologies for monitoring the workplace and general environment for the presence of mutagenic or carcinogenic pollutants, and (4) development of methodology for epidemiological investigations of human populations suspected or known to have been exposed to mutagenic or carcinogenic agents.

During this period, support was provided to the Environmental Mutagen Information Center located at the Oak Ridge National Laboratory. This permitted a literature data base on studies of chemical mutagens. Yearly bibliographies of research papers in the open literature were formulated.

Studies of the various project areas found support by Interagency Agreement with the Department of Energy for relevant work at the Oak Ridge National Laboratory. Also, contracts and grants were awarded directly to researchers at various universities in the United States and abroad.

Utilization of short-term tests for mutagenicity to screen environmental chemicals

A major development was the establishment of the Environmental Mutagenesis Test Development Program in the National Toxicology Program at NIEHS. This program started in 1979 and was designed for (1) development of an integrated program for routine testing of large numbers of chemicals for mutagenicity which included identification and development of new test systems, increased understanding of the mutagenesis process, test system validation, and development of a strategy for conducting large-scale testing and data evaluation and (2) implementation of a large-scale testing program capable of testing up to 1,000 chemicals per year in microbial mutagenicity assays. Contracts were awarded for testing using the

Beta-thalassemia homozygote and heterozygous sibs obtained in a biochemical specific locus experiment at the Research Triangle Institute.

Salmonella microsome assay, the Drosophila sex-linked recessive lethal assay and studies of chromosome aberrations and sister-chromatid exchange in Chinese hamster ovary cells as well as for operation of a chemical repository, and for the operation of a computerized data base management system.

By 1983 two laboratories conducted tests using the Salmonella microsome assay at a rate of 200 chemicals/year; three laboratories used the Drosophila sex-linked recessive lethal assay on 40 chemicals/year. Three laboratories under contract to conduct tests for cytogenetic damage and sister-chromatid exchange in Chinese hamster ovary cells now test chemicals at a rate of 100 chemicals/year. Two laboratories using the mouse lymphoma L5178Y cell culture system to test for induced gene mutations test at a rate of 50 chemicals/year. Short-term test data were compiled on more than 1,000 chemicals by 1984. One factor that entered into the selection of chemicals for testing was acquisition of genetic toxicology data on chemicals selected for chronic rodent toxicity studies and on chemicals which showed no evidence of carcinogenicity in rodents.

Support of investigations which serve to improve our ability to make human-risk estimations.

In 1981 in the National Toxicology Program two new research efforts provided data on the chemical induction of heritable specific gene mutations in mouse germ cells. Model mutagens (e.g., ethyl nitrosourea) and environmentally significant chemicals are tested at the Oak Ridge National Laboratory in the morphological specific locus assay and at the Research Triangle Institute in the biochemical specific locus assay. Tests for induced mouse heritable translocations will be carried out primarily on chemicals tested in the specific locus assays.

Chemicals currently under test include ethylene oxide, ethylene dibromide, hexamethylphosphoramide, urethane, and dibromochloropropane. By 1984 chemicals tested which gave negative results in specific locus tests were ethylene oxide, hexamethylphosphoramide, urethane, and dibromochloropropane. Ethylene oxide induced high frequencies of heritable translocations but the other three compounds showed no evidence of induced chromosomal damage in mouse germ cells. Ethylene dibromide, 6-mercaptopurine, and benzene are on test.

Development of assay systems for aneuploidy for use in mass-screening programs.

An NIEHS-sponsored workshop on Systems To Detect Induction of Aneuploidy by Environmental Mutagens was held in Savannah, Georgia, November 5-8, 1978. The workshop was organized to focus attention on a genetic event which contributes substantially to human genetic disease. These were largely ignored in the development of assay systems and mutagen screening programs. A number of promising systems being developed for use as assays for induced aneuploidy were discussed. There was concurrence by participants on the need for collaborative studies to expedite the development and validation of these systems.

In 1981, aneuploidy assays developed under contract in the fruit fly Drosophila and in the yeast Saccharomyces. By 1984 a three-part initiative was undertaken by NIEHS and EPA to promote further research on aneuploidy assays: (1) All of the available literature on aneuploidy assays and chemically induced aneuploidy is being critically evaluated by expert panels. Reports of these panels are to be published in *Mutation Research*; (2) an international collaborative study is being organized to evaluate currently available assays using a series of compounds known or suspected to induce aneuploidy; and (3) a symposium was held March 25-28, 1985, in Washington, D.C. to review and discuss aneuploidy and its impact on human health: mechanisms, assay systems, and chemicals that induce it.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
John E. Casida University of California Berkeley, CA	Mutagenic pesticide metabolites, photoproducts and impurities
Russell O. Sinnhuber Oregon State University Corvallis, OR	Metabolism and carcinogenicity of aflatoxins

Investigator and Institution	*Title*
Gerald M. Wogan Massachusetts Institute of Technology Cambridge, MA	Mutagenic activity of nitrosamines
Frederik H. Sobels University of Leiden Leiden, The Netherlands	Mutation induction by genotoxic agents
Karl G. Lark University of Utah Salt Lake City, UT	Use of plant cell culture to study genetic variation
Gerald N. Wogan Massachusetts Institute of Technology Cambridge, MA	Genetic toxicology testing
Charles C. Tong American Health Foundation Valhalla, NY	Comparative genetic effects of environmental chemicals
Michael J. Plewa University of Illinois Urbana, IL	Maize monitor for environmental genotoxins
John H. Burki University of California Berkeley, CA	Alkylating carcinogen mutagenesis in mammalian cells
Eric Eisenstadt Harvard University Boston, MA	Mutagenicity and metabolism of cyclopenta (C,D) pyrene
Thomas D. Stamato Wistar Institute Philadelphia, PA	Isolating carcinogen sensitive mammalian cell mutants
William R. Lee Louisiana State University Baton Rouge, LA	Genetic effect of alkylation in repair deficient oocytes
Edmond J. LaVoie Naylor Dana Institute Valhalla, NY	Quinolines: environmental mutagens and carcinogens
Reggie H. Stevens University of Iowa Iowa City, IA	Radiation-equivalency of mutagens/carcinogens
Darrel P. Falk Syracuse University Syracuse, NY	Environmental chemicals and chromosome breakage
James M. Gentile Hope College Holland, MI	*In vitro* activation of promutagens by green plants
Steven M. D'Ambrosio Ohio State University Columbus, OH	DNA damage following exposure to genotoxins

Investigator and Institution	*Title*
Leland L. Smith University of Texas Medical Branch Galveston, TX	Environmentally induced mutagenicity
William R. Lee Louisiana State University Baton Rouge, LA	Quantitative genetic study of environmental mutagens
Louise Prakash University of Rochester Rochester, NY	Alkylating agents: DNA damage, repair, and mutagenesis
Thomas D. Stamato Wistar Institute Philadelphia, PA	Mutagen induction of sectored colonies in CHO cells
Michael J. Smerdon Washington State University Pullman, WA	Carcinogen damage and repair of DNA in human chromatin
John H. Peters SRI International Menlo Park, CA	Food-derived promutagens/procarcinogens
Michael S. Esposito University of California Berkeley, CA	Genetic effects of carcinogens in mitosis and meiosis
Leonard F. Bjeldanes University of California Berkeley, CA	Mutagens, carcinogens produced by Fusarium moniliforme
Herbert S. Rosenkranz Case Western Reserve University Cleveland, OH	Studies on a class of ubiquitous environmental mutagens
Hebert S. Rosenkranz Case Western Reserve University Cleveland, OH	Nitroarenes: mutagenicity and DNA adducts
Robert T. Taylor Lawrence Livermore National Laboratory Livermore, CA	Metal methylation and mutagenesis
Nahum J. Duker Temple University Philadelphia, PA	Molecular basis for inhibition of DNA repair initiation
James M. Lavelle University of Connecticut Storrs, CT	Interactions between metals and non-metal mutagens
John D. Wilson Virginia Commonwealth University Richmond, VA	Modelling cellular response to genotoxic agents
Guylyn R. Warren Montana State University Bozeman, MT	Interactions of pollutants: effects on genetic toxicity

Investigator and Institution	*Title*
David Trkula Baylor College of Medicine Houston, TX	The role of herpesvirus thymidine kinase in mutagenesis
Mary E. Gaulden University of Texas Dallas, TX	Grasshopper neuroblast mutagen test system
Steven M. D'Ambrosio Ohio State University Columbus, OH	Genotoxicity in human fetal brain, dermis, and kidney cells
Eric Moon-Shong Tang University of Texas System Cancer Center Houston, TX	Repair of DNA damaged by model environmental chemicals
Lawrence Grossman Johns Hopkins University Baltimore, MD	Human DNA repair genes DNA repair enzymes in cells mutating antibody genes
Eric Eisenstadt Harvard University Boston, MA	Mutagenic specificity of environmental carcinogens
Philip E. Hartman Johns Hopkins University Baltimore, MD	Detection of environmental mutagens and antimutagens
Michael Karin University of Southern California Los Angeles, CA	Interactions of heavy metal ions with the human genome
Seymour Abrahamson University of Wisconsin Madison, WI	Induced mutation in germinal stem cells
Paul R. Gladstone Genetic Systems Corp. Seattle, WA	Rapid methods to monitor genetic damage in humans
Mary K. Conner University of Pittsburgh Pittsburgh, PA	A model for persistence of SCE lesion
Joseph B. Guttenplan New York University New York, NY	Mechanisms of carcinogenesis and mutagenesis
Subodh Kumar SUNY – College at Buffalo Buffalo, NY	Mutagenicity studies with dibenz (A,H) acridine derivative
William R. Lee Louisiana State University Baton Rouge, LA	Mechanisms of mutagenesis for environmental mutagens
Howard G. Shertzer University of Cincinnati Cincinnati, OH	Protection from environmental toxins/mutagens by indoles

Investigator and Institution	*Title*
Michael S. Risley Cornell University New York, NY	*In vitro* spermatogenic cell genotoxicity tests
Howard G. Gratzner University of Miami Miami, FL	*In vitro* and *in vivo* mutagenesis testing by flow cytometry
Timothy Jorgensen Johns Hopkins University Baltimore, MD	Radiation-induced DNA damage and repair at low doses
Avram Gold University of North Carolina Chapel Hill, NC	Activation of polycyclic environmental mutagens
Ronald Yasbin University of Rochester Rochester, NY	Excision repair in *bacillus subtilis*
Philip M. Iannaccone Northwestern University Chicago, IL	Reproductive toxicology of environmental mutagens
Stephen E. Bloom Cornell University Ithaca, NY	Embryos for detecting environmental mutagens
Dennis P. Smith Southern Methodist University Dallas, TX	Environmental mutagenesis and DNA repair

BIBLIOGRAPHY

Information Exchange

Bishop, J.B. and J.S. Wassom. *Toxicological Review of Busulfan* (Myleran) (in preparation).

Bradley, M.O., B. Bhuyan, M.W. Francis, R. Langenbach, A.R. Peterson, and E. Huberman. Mutagenesis by Chemical Agent in V-79 Chinese Hamster Cells: A Review and Analysis of the Literature: A Report of the Gene-Tox Program. *Mutat. Res. 87*:81-142, 1981.

Brockman, H.E., F.J. de Serres, T-M. Ong, D.M. DeMarini, A.J. Katz, A.J.F. Griffiths, and R.S. Stafford. Mutation Tests in *Neurospora crassa*. A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res. 133*:87-134, 1984.

Brown, M.M., J.S. Wassom, H.V. Malling, M.D. Shelby, and E.S. Von Halle. A Literature Survey of Bacterial, Fungal, and *Drosophila* Assay Systems Used in the Evaluation of Selected Chemical Compounds for Mutagenic Activity. *J. Natl. Cancer Inst. 62(4)*:841-971, April 1979.

Brusick, D., J. Ashby, F. de Serres, P. Lohman, T. Matsuhima, B. Matter, M. Mendelsohn, M. Waters, and J. Wassom. ICPEMC Committee I Interim Report, *Proposed Scheme for Evaluation and Interpretation of Short-Term Results* (in preparation).

Brusick, D., B.J. Kilbey, J. Ashby, H. Bartsch, U.H. Ehling, L. Kada, H.V. Malling, A.T. Natarajan, G. Obe, H.S. Rosenkranz, L.B. Russell, J. Schoneich, A.G. Searle, E. Vogel, J.S. Wassom, and F.K. Zimmermann. Screening Strategy for Chemicals that are Potential Germ-Cell Mutagens in Mammals. *Mutat. Res. 114*:117-177, 1983.

Brusick, D., V.F. Simmon, H.S. Rosenkranz, V.A. Ray, and R.S. Stafford. An Evaluation of the *Escherichia coli* WP2 and WP2 *uvr*A Reverse Mutation Assay. *Mutat. Res. 76*:169-190, 1980.

Caton, G.M., D.C. Michelson, B.N. Collier, J.B. Cooper, G.S. Danford, L.M. Frogge, R.J. Hamley, R.A. McDuffie, and P.T. Owen. *Inventory of Advanced Energy Technologies and Energy Conservation Research and Development: 1976-1978*. 3 Vols., Serials C, D, and E, Committee on Science and Technology, U.S. House of Representatives, Ninety-sixth Congress, January 1979, 3502 pp.

Caton, G.M., D.C. Michelson, B.N. Collier, J.B. Cooper, G.S. Danford, L.M. Frogge, R.J. Hamley, R.A. McDuffie, and P.T. Owen. *Inventory of Coal Research and Development*. Vol. 1, ORNL/EIS-120/V1, May 1979, 381 pp.

Caton, G.M., D.C. Michelson, B.N. Collier, J.B. Cooper, G.S. Danford, L.M. Frogge, R.J. Hamley, R.A. McDuffie, and P.T. Owen. *Inventory of Coal Research and Development*. Vol. 2, ORNL/EIS-120/V2, May 1979, 789 pp.

Clive, D., R. McCuen, J.F.S. Spector, C. Piper, and K.H. Mavournin. Specific Gene Mutations in L5178Y Cells in Culture. A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res. 115*:225-251, 1983.

Constantin, M.J. and E.T. Owens. Introduction and Perspectives of Plant Genetic and Cytogenetic Assays: A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* 99:1-12, 1982.

Francis, M.W. Two New Computerized Files at EMIC: *The Animal Information File and the Cell Transformation File*. Proceedings Environmental Mutagen Society 12th Annual Meeting, San Diego, CA, March 5-9, 1981.

Generoso, W.M., J.B. Bishop, D.G. Gosslee, G.W. Newell, C.J. Sheu, and E.S. Von Halle. Heritable Translocation Test in Mice. *Mutat. Res. 76*:191-215, 1980.

Green, S., A. Auletta, J. Fabricant, R. Kapp, M. Manandhar, C.J. Sheu, J. Springer, and B. Whitfield. Current Status of Bioassays in Genetic Toxicology — the Dominant Lethal Assay. A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* (in press).

Heddle, J.A., M. Hite, B. Kirkhart, K. Mavournin, J.T. MacGregor, G.W. Newell, and M.F. Salamone. The Induction of Micronuclei as a Measure of Genotoxicity. A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res. 123*:61-118, 1983.

Heidelberger, C., A.E. Freeman, R.J. Pienta, A. Sivak, J.S. Bertram, B.C. Casto, V.C. Dunkel, M.W. Francis, T. Kakunaga, J.B. Little, and L.M. Schechtman. Cell Transformation by Chemical Agents — A Review and Analysis of the Literature. Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res. 114*:283-385, 1983.

Hsie, A.W., D.A. Casciano, D.B. Couch, D.F. Krahn, J.P. O'Neill, and B.L. Whitfield. The Use of Chinese Hamster Ovary Cells to Quantify Specific Locus Mutation and to Determine Mutagenicity of Chemicals. *Mutat. Res.* 86:193-214, 1981.

Kafer, E.S., B.R. Scott, G.L. Dorn, and R.S. Stafford. *Aspergillus nidulans*: Systems and Results of Tests for Chemical Induction of Mitotic Segregation and Mutation. I. Diploid and Duplication Assay System: A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* 98:1-48, 1982.

Kier, L.D., D.J. Brusick, A.E. Auletta, E.S. Von Halle, V.F. Simmon, M.M. Brown, V.C. Dunkel, J. McCann, K. Mortelmans, M.J. Prival, T.K. Rao, and V.A. Ray. The *Salmonella typhimurium*/Microsome Mutagenicity Assay. A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* (in press).

Larsen, K.H. Indexed Collection of the Literature on Aneuploidy. *Environ. Health Perspect. 31*:167, August 1979.

Larsen, K.L., D. Brash, J.E. Cleaver, R.W. Hart, V.M. Maher, R.B. Painter, and G.S. Sega. DNA Repair Assay as Tests for Environmental Mutagens: A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* 98:287–318, 1982.

Latt, S.A., J. Allen, S.E. Bloom, A.V. Carrano, E. Falke, D. Kram, E. Schneider, R. Schreck, R. Tice, B.L. Whitfield, and S. Wolff. Sister Chromatid Exchanges: A Report of the Gene-Tox Program. *Mutat. Res. 87*:17–62, 1981.

Lee, W.R., S. Abrahamson, R. Valencia, E.S. Von Halle, F.E. Wurgler, and S. Zimmering. The Sex-Linked Recessive Lethal Test for Mutagenesis in *Drosophila melanogaster*. Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res. 123*:183–279, 1983.

Legator, M.S., E. Bueding, R.P. Batzinger, T.H. Connor, E. Eisenstadt, M.G. Farrow, A. Ficsor, A.W. Hsie, J. Seed, and R.S. Stafford. An Evaluation of the Host-Mediated Assay and Body Fluid Analysis: A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* 98:319–374, 1982.

Leifer, Z.T., M. Mandel, E. Zeiger, R.S. Stafford, and H.S. Rosenkranz. An Evaluation of Tests Using DNA Repair-Deficient Bacteria for Predicting Genotoxicity and Carcinogenicity: A Report of the U.S. EPA's Gene-Tox Program. *Mutat. Res. 87*:211–297, 1981.

Loprieno, N., R. Barale, E.S. Von Halle, and R.C. von Borstel. Testing of Chemicals for Mutagenic Activity with *Schizosaccharomyces pombe*. A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res. 115*:215–223, 1983.

Miller, I.C. Environmental Mutagen Information Center. *EMIC Newsletter (1)*, fall 1979.

Miller, I.C. Environmental Mutagen Information Center. *EMIC Newsletter*, winter 1981/1982.

Mitchell, A.D., D.A. Casciano, M.L. Meltz, D.E. Robinson, R.H.C. San, G.M. Williams, and E.S. Von Halle. Unscheduled DNA Synthesis Tests. A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res. 123*:363–410, 1983.

Owens, E.T. New Fields in the Environmental Mutagen Information (EMI) Data Base. *DOE/RECON Newsletter 3*:6–11, 1979.

Popp, D.M. and M.W. Francis. Age-Associated Changes in T-B Cell Cooperation Demonstrated by the Allogenic Effect. *Mech. Aging Develop. 10*:341–353, 1979.

Preston, R.J., W. Au, M.A. Bender, J.G. Brewen, A.V. Carrano, J.A. Heddle, A.F. McFee, S. Wolff, and J.S. Wassom. Mammalian *In Vivo* and *In Vitro* Cytogenetic Assays: A Report of the U.S. EPA's Gene-Tox Program. *Mutat. Res. 87*:143–188, 1981.

Ray, V., L.D. Kier, A.E. Auletta, K.L. Kannan, S. Nesnow, and M.D. Waters. An Approach to Identifying Specialized Batteries of Bioassays for Specific Classes of Chemicals: Class Analysis Using Mutagenicity and Carcinogenicity Relationships and Phylogenetic Concordant and Discordant Patterns. Part 1. Composition and Analysis of the Overall Data Base. A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* (in press).

Russell, L.B., C.S. Aaron, F. de Serres, W.M. Generoso, K.L. Kannan, M. Shelby, J. Springer, and P. Voytek. Evaluation of Mutagenicity Assays for Purposes of Genetic Risk Assessment. A Report of the U.S. Environmental Protection Agency Gene-Tox Program *(in preparation)*.

Russell, L.B., P.B. Selby, E. Von Halle, W. Sheridan, and L. Valcovic. The Mouse Specific-Locus Test with Agents Other than Radiations: Interpretation of Data and Recommendations for Future Work. *Mutat. Res. 86*:329–354, 1981.

Russell, L.B., P.B. Shelby, E. Von Halle, W. Sheridan, and L. Valcovic. Use of the Mouse Spot Test in Chemical Mutagenesis: Interpretation of Data and Recommendations for Future Work. *Mutat. Res. 86*:355–379, 1981.

Scott, B.R., G.L. Dorn, E. Kafer, and R.S. Stafford. *Aspergillus nidulans*: System and Results of Tests for Induction of Mitotic Segregation and Mutation. II. Haploid Assay Systems and Overall Response of 11 Systems. A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* 98:49–94, 1982.

Valencia, R., S. Abrahamson, W.R. Lee, E.S. Von Halle, R.C. Woodruff, F.E. Wurgler, and S. Zimmering. Chromosome Mutation Tests for Mutagenesis in *Drosophila melanogaster*. A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res. 134*:61–88 (1984).

Wassom, J.S. The Environmental Mutagen Information Center and the Environmental Teratology Information Center. *IRPTC Bull. 2(2)*:6–7, July 1979.

Wassom, J.S. Mutagenicity Research in the United States. *Mutagens Toxicol.* 9:4–15, 1980.

Wassom, J.S. Mutagenesis, Carcinogenesis, and Teratogenesis Information Systems. In: *Safe Handling of Chemical Carcinogens, Mutagens, and Teratogens*, Vol. 1, Chap. 16, D.G. Walters (ED), Ann Arbor Science Publishers, Inc., Ann Arbor, MI, 1980, 299–311.

Wassom, J.S. The Storage and Retrieval of Chemical Mutagenesis Information. In *Progress in Environmental Mutagenesis*, M. Alacevic (ED), Elsevier/North Holland Biomedical Press, Amsterdam, 1980, 313–330.

Wasson, J.S. and V.H. Frankos. The Environmental Teratology Information Center's Data Extraction Project. *Teratology 25(2)*:80A, 1982.

AVAILABLE

Wassom, J.S., E.S. Von Halle, B.L. Whitfield, R.S. Stafford, K.H. Larsen, M.W. Francis, I.C. Miller, M.A. Davidson, L. Huberman, S.Y. Uppuluri, R.T. Haas, and M.M. Brown. *Chemical Mutagenesis: A Survey of the 1975–1976 Literature*. ORNL/EMIC-10, October 1979, 362 pp.

Wyrobek, A.J., L.A. Gordon, J.G. Burkhart, M.W. Francis, R.W. Kapp, Jr., G. Letz, H.V. Malling, J.C. Topham, and M.D. Whorton. An Evaluation of Human Sperm as Indicators of Chemically Induced Alterations of Spermatogenic Function. A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res. 115*:73–148, 1983.

Wyrobek, A.J., L.A. Gordon, J.G. Burkhart, M.W. Francis, R.W. Kapp, Jr., G. Letz, H.V. Malling, J.C. Topham, and M.D. Whorton. An Evaluation of the Mouse Sperm Morphology Test and Other Sperm Tests in Nonhuman Mammals. A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res. 115*:1–72, 1983.

Zimmermann, F.K., R.C. von Borstel, E.S. Von Halle, J.M. Parry, D. Siebert, G. Zetterberg, R. Baraley, and N. Loprieno. Testing of Chemicals for Genetic Activity with *Saccharomyces cerevisiae*: A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res. 133*:199–244, 1984.

Manuscripts in Preparation for Other Journals

Wassom, J.S. The Use of Selected Toxicological Information Resources in Assessing Relationships Between Chemical Structure and Biological Activity (in preparation for submission to *Environmental Health Perspectives*).

Books and Book Chapters

Von Halle, E.S. An Appendix To Be Published as Part of the International Program for Chemical Safety (IPCS) Workshop Proceedings on Short-Term Tests for Genotoxicity and Carcinogenicity. *A Tabular Review of the Published Mutagenicity Literature for IPCS Study Compounds*.

Wassom, J.S. and S.Y. Uppuluri. *The Use of Information Resources in the Evaluation of Agents for Carcinogenicity*. In: Y. Hayashi and T. Kuroki (EDS), New Mechanism and Evaluation of Cancer (1984), Science Forum, Tokyo, Japan.

Other Publications

Mavournin, K.H. *Comparison of Aneuploidy with Other Genetic End Points*. Proceedings of EPA Workshop on Approaches for Assessing the Risk of Aneuploidy Induction by Chemicals, Washington, D.C., October 5–6, 1983.

Development of Test Systems To Screen Chemicals For Mutagenic Activity

Dunkel, V.C., E. Zeiger, D. Brusick, E. McCoy, D. McGregor, K. Mortelmans, H.S. Rosenkranz, and V.F. Simmon. Reproducibility of Microbial Mutagenicity Assays: I. Test with *Salmonella typhimuirum* and *Escherichia coli* using a Standardized Protocol. *Environ. Mutag. 6* (Suppl. 2):1–252, 1984.

Frezza, D., B. Smith, E. Zeiger. The Intrasanguineous Host Mediated Assay Procedure Using *Saccharomyces cerevisiae:* Comparison with Two Other Metabolic Activation Systems. *Mutat. Res. 108*:161–168, 1983.

Margolin, B.H., N. Kaplan, E. Zeiger. *Statistical Analysis of the Ames Salmonella/Microsome Test*, Proc. Natl. Acad. Sci. 78(6):3779–3783, 1981.

Pagano, D.A., E. Zeiger. Suppressive Effects of Chemicals in Mixture on the Salmonella Plate Test Response in the Absence of Apparent Toxicity (Abstract). *Environ. Mutag. 5*:473–474, 1983.

Rowleg, R.M., E. Zeiger, C. Russell. A Prophet-Based Laboratory Data Entry and Data-Base Management System for the Environmental Mutagenesis Test Development Program. *Computers Biomed Res. 15(6)*:544–554, 1982.

Schairer, L.A. and R.C. Sautkulis. Detection of Ambient Levels of Mutagenic Atmospheric Pollutants with the Higher Plant Tradescantia. In: E.J. Klekowski (ED) *Environmental Mutagenesis, Carcinogenesis, and Plant Biology, Vol. 2*, 153–194, Praeger Scientific, New York, 1982.

Thompson, M. Emmerling- and M.M. Nawrocky. Evidence of Gametic Mutation for Flower Color in Tradescantia. *Environ. and Expt'l. Botany 22*:403–408, 1982.

Thompson, M. Emmerling- and M.M. Nawrocky. Genetic Basis for Using Tradescantia Clone 4430 as an Environmental Monitor of Mutagens. *J. Heredity 71*:261–265, 1980.

Van't Hof, J. and L.A. Schairer. Tradescantia Assay System for Gaseous Mutagens. *Mutat. Res. 99*:303–315, 1982.

Woodruff, R.C., J.M. Mason, R. Valencia, S. Zimmering. Chemical Mutagenesis Testing in Drosophila: I. Comparison of Positive and Negative Controls for Sex-Linked Recessive Lethal Mutations and Reciprocal Translocations in Three Laboratories, *Environ. Mutagenesis 6*:189–202, 1984.

Zeiger, E. Genetic Toxicity Tests and the National Toxicology Program. *Ann. NY Acd. Sci. 407*:387–394, 1983.

Zeiger, E., J.W. Drake. An Environmental Mutagenesis Test Development Programme. In: Molecular and Cellular Aspects of Carcinogen Screening Tests, Montsano, R., et al, Lyon, Fr., pp. 303–313, *IARC Sci. Pub. No. 27*: 1980.

Zeiger, E., D.A. Pagano. Suppression of Mutagenic Responses in the Salmonella Plate Test by Substances Affecting the Target Bacteria (Abstract), *Environ. Mutagen 4*:414–415.

Zeiger, E., D.A. Pagano. Suppressive Effects of Chemicals in Mixture on the Salmonella Plate Test Response in the Absence of Apparent Toxicity, *Environ. Mutag.* (in press).

Zeiger, E., D. Pagano, I.C.G. Robertson. Rapid and Simple Scheme for Confirmation of Salmonella Tester Strain Phenotype. *Environ. Mutagen 3(3)*:205–209, 1981.

Zimmering, S., J.M. Mason, R. Valencia, R.C. Woodruff. Chemical Mutagenesis Testing in Drosophila. II. Results of 20 Coded Compounds Tested for the National Toxicology Program, *Environ. Mutag.* (in press).

Test Method Validation and Utilization

Battaglia, G., C. Wood, D. Stuermer, and J.S. Felton. Analysis of Cooked Food Mutagens by Gas Chromatography. *J. Ag. and Food Chem.*, in press.

Bjeldanes, L.F., J.S. Felton, and F.T. Hatch. Mutagens in Cooked Food. Chapter 9 In: *Xenobiotics in Foods and Feeds*, J. W. Finley and D.E. Schwass (EDS), ACS Symposium Series 234, American Chem. Soc. Publ., Washington, DC, 149–168, 1983.

Bjeldanes, L.F., J.S. Felton, and F.T. Hatch. Mutagens in Cooked Foods: Chemical Aspects. In: *Nutritional and Metabolic Aspects of Food Safety, Advances in Experimental Medicine and Biology*, M. Friedman (ED), Plenum Press, NY, Vol. 177, 545-554, 1984.

Bjeldanes, L.F., R.K. Grose, P.H. Davis, D.H. Stuermer, S.K. Healy, and J.S. Felton. An XAD-2 Resin Method for Efficient Extraction of Mutagens from Fried Ground Beef. *Mutat. Res. Lett. 105*:43–49, 1982.

Bjeldanes, L.F., M.M. Morris, H. Timourian, and F.T. Hatch. Effects of Meat Composition and Cooking Conditions on Mutagen Formation in Fried Ground Beef. *J. Agr. and Food Chem. 31*:18–21, 1983.

Bjeldanes, L.F., M. Morris, J.S. Felton, S. Healy, D. Stuermer, P. Berry, H. Timourian, and F.T. Hatch. Formation of Mutagens During Cooking of Protein Foods. *Environ. Mut. 3*:349–350, 1981.

Bjeldanes, L.F., M.M. Morris, J.S. Felton, S. Healy, D. Stuermer, P. Berry, H. Timourian, and F.T. Hatch. Mutagens from the Cooking of Food II. Survey by Ames/Salmonella Test of Mutagen Formation in the Major Protein-Rich Foods of the North American Diet. *Food and Chem. Toxicol.*, *20*:357-363, 1982.

Bjeldanes, L.F., M.M. Morris, J.S. Felton, S. Healy, D. Stuermer, P. Berry, H. Timourian, and F.T. Hatch. Mutagens from the Cooking of Food III. Survey by Ames/Salmonella Test of Mutagen Formation in Secondary Sources of Cooked Dietary Protein. *Food and Chem. Toxicol.*, *20*:365-369, 1982.

Brockman, H.E., C.Y. Hung, and F.J. de Serres. Effect of the *uvs-2* Allele on the Killing and Mutagenic Activities of Nitrous Acid in the *ad-3* Forward-Mutation Test in *Neurospora crassa. Environ. Mut. 4*:717, 1982.

Brockman, H.E., C.Y. Hung, and F.J. de Serres. Potent Mutagenicity of Formaldehyde in a Nucleotide Excision Repair-Deficient Heterokaryon of *Neurospora crassa. Env. Mut. Vol. 3*, 379-380, 1981.

Brockman, H.E., C.Y. Hung, T.M. Ong, and F.J. de Serres. Mutagenicity of TRENIMON and 2,3,5,6-tetraethylenmino-1, 4-benzoquinone (TEB) at the *ad-3* Region of *Neurospora crassa*. Abstract, *Env. Mut. 2*: 241, 1980.

Brockman, H.E., C.Y. Hung, F.J. de Serres, and T-M. Ong. Potent Mutagenicity of 6-N-Hydroxylamino Purine and 2-Amino-N^6-Hydroxyadenine at the *ad-3* region of *Neurospora crassa. Environ. Mut. 1*:133, 1979.

Brockman, H.E., F.J. de Serres, C.Y. Hung, and L.K. Overton. Mutagenicity of Toxaphene in the *ad-3* Forward-mutation Test in Nucleotide Excision Repair-deficient and proficient Dikaryons of *Neurospora crassa. Environmental Mutagenesis 5*:502, 1983.

Brockman, H.E., F.J. de Serres, Tong-Man Ong, D.M. DeMarini, A.J. Katz, A.J.F. Griffiths, and Robert S. Stafford. Mutation Tests in *Neurospora crassa*: A report of the U.S. Environmental Protection Agency Gene-Tox Program, *Mutation Research 113*:87–134, 1984.

Bronzetti, G., I.P. Lee, E. Zeiger, H.V. Malling, K. Suzuki. Genetic Effects of TCDD *In Vitro* and *In Vivo* Using D7 Strain of S. Cerevisiae (Abstract), *Mutat. Res 74(3)*:206–207, 1980.

Bronzetti, G., E. Zeiger, I.P. Lee, K. Suzuki, H.V. Malling. Genetic Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in Yeast *In Vitro* and *In Vivo*. In: Chlorinated Dioxins and Related Compounds, Pergamon Ser *Environ. Sci. 5*:429–436, 1982.

Dunkel, V.C., E. Zeiger, D. Brusick, E. McCoy, D. McGregor, K. Mortelmans, H.S. Rosenkranz, and V.F. Simmon. Reproducibility of Microbial Mutagenicity Assays: 1. Tests with Salmonella Typhimurium and Escherichia coli using a Standardized Protocol. *Environ. Mutag. 6 (Suppl 2)*:1–252, 1984.

Felton, J.S., L.F. Bjeldanes, and F.T. Hatch. Mutagens in Cooked Foods: Chemical Aspects. In: *Nutritional and Metabolic Aspects of Food Safety, Advances in Experimental Medicine and Biology*, M. Friedman (ED), Plenum Press, NY, Vol. 177, 555–566, 1984.

Felton, J.S., F.T. Hatch, M.G. Knize, and L.F. Bjeldanes. Mutagens in Cooked Beef: Characterization and Genotoxic Effects. In: *Diet and Cancer: From Basic Research to Policy Implications*, DA. Roe (ED), Alan R. Liss, Inc., New York, 177–194, 1983.

Felton, J.S. and S.K. Healy. Mutagenic Activation of Cooked Ground Beef by Human Liver Microsomes, *Mutat. Res.*, in press.

Felton, J.S., K. Healy, L. Avalois, and B. Wuebbles. Comparison of Mutagenicity of Two Cooking Mutagens with Three Microbial Bioassays. Presented at EMS Meeting, San Antonio, TX, Mar. 3-6, 1983. *Environ. Mut. 5*:446, 1983.

Felton, J., S. Healy, D. Stuermer, C. Berry, H. Timourian, F. Hatch, L. Bjeldanes, and M. Morris. Improved Isolation and

Characterization of Mutagenic Fractions from Cooked Ground Beef. *Environ. Mutag. 2*:303–304, 1980.

Felton, J.S., S.K. Healy, M. Knize, D.H. Stuermer, P.W. Berry, H. Timourian, F.T. Hatch, L.F. Bjeldanes, and M. Morris. Metabolism of Mutagenic Fractions from Cooked Ground Beef. J. Supramol. *Struct. Cell Biochem. [Suppl.] 5*:166, 1981.

Felton, J.S., S.K. Healy, M. Knize, D.H. Stuermer, P.W. Berry, H. Timourian, F.T. Hatch, M. Morris, and L.F. Bjeldanes. *In Vitro* Human and Rodent Metabolism of Mutagenic Fractions from Cooked Ground Beef. *Environ. Mut. 3*:342, 1981.

Felton, J.S., S.K. Healy, D.S. Orwig, D.H. Stuermer, P.W. Berry, H. Timourian, F.T. Hatch, L.F. Bjeldanes, and M. Morris. Metabolism of Mutagenic Fraction from Cooked Ground Beef. *Cancer Res. 21*:125, 1980.

Felton, J.S., S. Healy, D. Stuermer, C. Berry, H. Timourian, F.T. Hatch, M. Morris, and L.F. Bjeldanes. Mutagens from the Cooking of Food I. Improved Isolation and Characterization of Mutagenic Fractions From Cooked Ground Beef. *Mutat. Res. 88*:33–44, 1981.

Felton, J., M.C. Knize, C. Wood, B. Wuebbles, S.K. Healy, D.H. Stuermer, L.F. Bjeldanes, B.J. Kimble, and F.T. Hatch. Isolation and Characterization of New Mutagens from Fried Ground Beef. *Carcinogenesis 5*:95–102, 1984.

Generoso, W., M.D. Shelby, F.J. de Serres (EDS). *DNA Repair and Mutagenesis in Eukaryotes*, Plenum Press, New York, 1980.

Griesemer, R.A., A.G. Ulsmer, J.C. Arcos, J.R. Beall, A.E. Blair, T.F.X. Collins, F.J. de Serres, R.B. Everson, J.F. Gambil, D.W. Gaylor, D.H. Groth, H.K. Kang, R.A. Keenlyide, J.W. Lloyd, P. Nettesheim, V. Saffiotti, E.K. Weisburger. Report of the Federal Panel on Formaldehyde: Mutagenicity, *Environmental Health Perspectives, Vol. 43*:139–168, 1982.

Grose, K.R., I. Kim, and L.F. Bjeldanes. Deuteration of Mutagenic Aromatic Nitrogen Heterocycles Derived from Protein and Amino Acid Pyrolysates. *J. Agr. and Food Chem. 30*:766–768, 1982.

Guthrie, J., I.G.C. Robertson, E. Zeiger, J.A. Boyd, T.E. Eling. Selective Activation of Some Dihydrodiols of Several Polycyclic Aromatic Hydrocarbons to Mutagenic Products by Prostaglandin Synthetase, *Cancer Res. 42*:1620–1623, 0000.

Hatch, F.T. Report of Mini-Workshop on Genetic Toxicology and Food Mutagens. *Environmental Mutagenesis and Carcinogenesis Panel, U.S.-Japan Cooperative Medical Sciences Program*, Livermore, CA, November 1981.

Hatch, F.T., J.S. Felton, and L.F. Bjeldanes. Mutagens from the Cooking of Food: Thermic Mutagens in Beef. In: *Carcinogens and Mutagens in Food, Critical Reviews of Toxicology.* H.F. Stich (ED), CRC Press, Boca Raton, FL, 147–163, 1982.

Hatch, F.T., J.S. Felton, S. Healy, D. Stuermer, P. Berry, and H. Timourian. Formation of Mutagens During Cooking of Protein Foods. J. Supramol. *Struct. Cell Biochem. [Suppl.] 5*:192, 1981.

Hatch, F.T., J.S. Felton, D.H. Stuermer, and L.F. Bjeldanes. Identification of Mutagens from the Cooking of Food. In: *Chemical Mutagens: Principles and Methods for Their Detection*, Vol. 9, Chapter 6, F.J. de Serres (ED), Plenum Publishing Corporation, New York, in press.

Hatch, F.T., J.S. Felton, L.H. Thompson, A.V. Carrano, S.K. Healy, E.P. Salazar, and J.L. Minkler. Comparative Genotoxicity of the Food Mutagens Trp-P-2 and IQ in Salmonella and CHO Cells. *Environ. Mut. 4*:368, 1982.

Haworth, S.R., T.E. Lawlor, K. Mortelmans, W. Speck, E. Zeiger. Salmonella Mutagenicity Test Results for 250 Chemicals. *Environ. Mutag. 5 (Suppl 1)*:3–142, 1983.

Haworth, S.R., T.E. Lawlor, E. Zeiger. Salmonella Mammalian-Microsome Preincubation Mutagenicity Assays of 113 Chemicals Using Both Aroclor 1254 Induced Rat and Hamster S-9. *Environ. Mutagen. 3*:324, 1981.

Hunt, C.L., D.B. Walters, E. Zeiger. Approaches for Safe Handling Procedures and Design of a High-Hazard Laboratory for Life Scientists. In: *Safe Handling of Chemical Carcinogens, Mutagens, Teratogens and Highly Toxic Substances, Volume I*, D.B. Walters (ED), Ann Arbor Science Inc., Ann Arbor, MI:31–47, 1980.

Knize, M.G., B. Wuebbles, and J.S. Felton. The Advantages of a Semimicro HPLC System for the Purification of Small Amounts of Biologically Active Material. *LC 2*:222–224, 1984.

Knize, M.G., B. Wuebbles, E. Fultz, M. Morris, R. Taylor, J. Felton, L. Bjeldanes, and F. Hatch. *Characterization of the Mutagens in Cooked Beef: Chromatographic Patterns at Different Temperatures and Cooking Conditions*. Presented at American Society of Biological Chemists, San Francisco, June 1983, Federation Proc. 42: 2089, 1983.

Margolin, B.H., K.J. Risko, M.D. Shelby, E. Zeiger. Sources of Variability in Ames Salmonella-Typhimurium Tester Strains—Analysis of the International Collaborative Study on Genetic Drift. *Mutat. Res. 130(1)*:11–25, 1984.

Matijasevic, Z. and E. Zeiger. *Mutagenicity of Pyrene in Salmonella (Abstract)*. Presentation—Genotoxicity and Environmental Mutagen Society—RTP, NC—10/14/83.

Minkler, J.L. and A.V. Carrano. *In Vivo* Cytogenetic Effects of the Cooked-Food-Related Mutagens Trp-P-2 and IQ in Mouse Bone Marrow. *Mutat. Res.*, in press.

Mohn, G.R., A.A. van Zeeland, Ada G.A.C. Knapp, B.W. Glickman, A.T. Natarajan, M. Brendel, F.J.de Serres, and C.S. Aaron. Quantitative Molecular Dosimetry of Ethylmethane Sulfonate (EMS) in Several Genetic Test Systems. In: *Shortterm Test Systems for Detecting Carcinogens*, K.H. Norpoth and R.C. Garner (EDS), Springer-Verlag, Berlin/Heidelberg, 160–169, 1980.

Moore, D. and J.S. Felton. A Microcomputer Program for Analyzing Ames Test Data. *Mutat. Res. Lett. 119*: 95–102, 1983.

Mortelmans, K., S. Haworth, W. Speck, and E. Zeiger. Mutagenicity Testing of Agent Orange Components and Related Chemicals. *Toxicol Appl. Pharmacol.* (in press).

Ong, T. and F.J. de Serres. Genetic Analysis of *ad-3* Mutants Induced by AF-2 and Two Other Nitrofurans in *Neurospora crassa. Environ. Mut. 1*:133, 1979.

Ong, T. and F.J. de Serres. Genetic Analysis of *ad-3* Mutants Induced by Hycanthone, Lucanthone and their Indazole Analogs in *Neurospora crassa. J. Env. Path. and Tox. 4*:1–8, 1980.

Ong, T. and F.J. de Serres. Genetic Analysis of *ad-3* Mutants Induced by AF-2 and Two Other Nitrofurans in *Neurospora crassa. Env. Mut. 3*:151–158, 1981.

Ong, T.M., Barbara Slade, and F.J. de Serres. Mutagenicity and Mutagenic Specificity of Metronidazole and Nirodazole in *Neurospora crassa. J. Environ. Path. Tox. 2*:1109–1118, 1979.

Overton, L.K., C.Y. Hung, H.E. Brockman, and F.J. de Serres. Mutagenic Potency and Specificity of 2-Amino-N^6-Hydroxyadenine in the *ad-3* Forward-Mutation Test in Nucleotide Excision Repair-deficient and -proficient Dikaryons of *Neurospora crassa. Environmental Mutagenesis 5*:501, 1983.

Pagano, D.A., M.D. Shelby. Mutagenicity of 4-Chloromethylbiphenyl in the Salmonella/Microsome Assay. *Mutat. Res. 100*:67–70, 1982.

Pagano, D.A., B. Yagen, O. Hernandez, J.R. Bend, E. Zeiger. Mutagenicity of (R) and (S) Styrene 7,8-Oxide and the Intermediary Mercapturic Acid Metabolites Formed from Styrene 7,8-Oxide. *Environ. Mutagen 4*:575–584, 1982.

Pagano, D., E. Zeiger, A.A. Nomeir. Mutagenic Structure Activity Studies on Tris (2,3-Dibromopropyl) Phosphate (Tris-BP) Metabolites (Abstract), *Environ. Mutagen. 3*:334, 1981.

Plumlee, C., L.F. Bjeldanes, F.T. Hatch. Food Item Priority Assessment for Studies of Mutagen Production During Cooking. *J. Am. Dietet. Assoc. 79*:201–204, 1981.

Resnick, M.A., J.C. Game, S. Stasiewicz. Genetic Effects of UV Irradiation on Excision-Proficient and -Deficient Yeast During Meiosis. *Genetics 104 (4)*:603–618, 1983.

Schairer, L.A., R.C. Sautkulis, and N.R. Tempel. Monitoring Ambient Air for Mutagenicity Using the Higher Plant Tradescantia. In: R.R. Tice, D.L. Costa, and K.M. Schaich (EDS), *Proceedings of Symposium on the Genotoxic Effects of Airborne Agents*, Brookhaven National Laboratory, February 9–11, 1981, 123–139, Plenum Press, New York, 1982.

Schairer, L.A., J. Van't Hof, C.G. Hayes, R.M. Burton, and F.J. de Serres. Measurement of Biological Activity of Ambient Air Mixtures Using a Mobile Laboratory for *in situ* Exposures: Preliminary Results from the Tradescantia Plant Test System. In: M.D. Waters (ED) *Symposium on Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures*, Williamsburg, VA, Feb. 21–23, 1978, Vol. 15, 421–440, Plenum Press, New York, 1978.

de Serres, F.J. Comparison of the Induction of Specific Locus Mutations in Wild-type and Repair Deficient Strains of *Neurospora crassa*. In: *Molecular and Cellular Mechanisms of Mutagenesis*. J.F. Lemont and W.M. Generoso (EDS). Plenum Press, New York, 335-343, 1982.

de Serres, F.J. Induction and Genetic Characterization of Specific Locus Mutations in the *ad-3* Region in Two-Component Heterokaryons of *Neurospora crassa*. In: Short-term tests for Chemical Carcinogens (EDS) H.F. Stich and R.H.C. San, New York, 175–186, 1981.

de Serres, F.J. Mutation-induction in Repair-Deficient Strains in Neurospora. In: *DNA Repair and Mutagenesis in Eukaryotes*, W. Generoso, M. D. Shelby, and F. J. de Serres (EDS), Plenum Press, NY, 75–84, 1980.

de Serres, F. J. Problems Associated with the Application of Short-term Tests for Mutagenicity in Mass-Screening Programs. *Environ. Mut. 1*:203–208, 1979.

de Serres, F.J. The Use of Neurospora in the Evaluation of the Mutagenic Activity of Environmental Chemicals, *Environmental Mutagenesis 5*:341-351, 1983.

de Serres, F.J., H.E. Brockman, and C.Y. Hung. Effects of Heterokaryotic State of the *uvs-2* Allele in *Neurospora crassa* on Mitomycin C-induced Killing and Mutation. *Env. Mut. 3*:397, 1981.

de Serres, F.J., H.E. Brockman, C.Y. Hung, and T.M. Ong. Comparison of the Mutagenic Effect of 2-Aminopurine and Actinomycin D in Excision-Repair Deficient and Wild-type Two-component Heterokaryons of *Neurospora crassa, Environ. Mut. 1*:132, 1979.

de Serres, F.J., C.Y. Hung, and H.E. Brockman. Molecular Dosimetry of the Chemical Ethyl Methanesulfonate: Quantitative Comparison of the Mutagenic Potency in *Neurospora crassa* and *Saccharomyces cerevisiae. Mutation Research 119*:45-54, 1983.

de Serres, F.J., H. Inoue, and M.E. Schupbach. Induction of *ad-3* Mutants in Nucleotide Excision-repair-deficient Strains Provides Evidence for Qualitative Differences in the Spectrum of Genetic Alterations from that found in the Wild-type Strain of *Neurospora crassa. Environmental Mutagenesis 5*:501, 1983.

de Serres, F.J. and H.V. Malling. The Role of Neurospora in Evaluating Chemicals for Mutagenic Activity, Ann. N.Y. Acad. *Sci., 407*:177–185, 1983.

de Serres, F.J., H.V. Malling, H.E. Brockman and C.Y. Hung. Mutagenicity of Epichlorohydrin and 1,2-Dibromoethane in Heterokaryon 12 of *Neurospora crassa*. *Environ. Mut.* *4*:398–399, 1982.

de Serres, F.J. and M.D. Shelby (EDS). *Comparative Chemical Mutagenesis*, Plenum Press, New York, 1981.

Shelby, M.D., S. Stasiewicz. Chemicals Showing No Evidence of Carcinogenicity in Long-term, Two-species Rodent Studies: Their Use in Short-term Test Evaluations. *Environ. Mutag.* *6*:871–878, 1984.

Speck, W.T., L.W. Meyer, E. Zeiger, H.S. Rosenkranz. Mutagenicity and DNA-Modifying Activity of 2-Nitropropane. *Mutat. Res. 104*:49–54, 1982.

Taylor, R.T., E. Fultz, and M. Knize. Purification and Identification of Three Heterocyclic Amine Mutagens-carcinogens in a Boiled Beef Supernatant System. *Biochem. Biophys. Res. Commun.*, in press.

Taylor, R.T., E. Fultz, and V. Shore. Food Mutagen Formation in Model Boiling Systems. *Environ. Mut. 3*:349, 1981.

Taylor, R.T., E. Fultz, and V. Shore. Mutagen Formation in a Model Beef Boiling System I. Conditions with a Soluble Beef-Derived Fraction. *J. Env. Sci. and Health, A. Env. Sci. and Eng*, in press.

Taylor, R.T., V. Shore, and E. Fultz. Mutagen Formation in a Model Beef Boiling System II. Effects of Proteolysis and Comparison of Soluble Fractions from Several Protein Sources. *J. Env. Sci. and Health, A. Env. Sci. and Eng*, in press.

Taylor, R.T., V. Shore, and E. Fultz. Mutagen Formation in a Model Beef Boiling System: Stimulation Studies with Amino Acids and Other Agents. *Environ. Mut. 4*:368, 1982.

Thompson, L.H., A.V. Carrano, E. Salazar, J.S. Felton, and F.T. Hatch. Comparative Genotoxic Effects of the Cooked-Food-Related Mutagens Trp-P-2 and IQ in Bacteria and Cultured Mammalian Cells. *Mutat. Res. 117*:243–257, 1983.

Wieland, J., M. Bender, and M.A. Resnick. The Effects of Mitomycin C and X-rays on the Efficiency of DNA Mediated Cell Transformation (Abstract), Proc. 4th Ann. Congress for *Recombinant DNA Res.*—San Diego, CA.

van Zeeland, A.A., G.R. Mohn, C.S. Aaron, B.W. Glickman, M. Brendel, F. J. de Serres, H.E. Brockman, and C.Y. Hung. Molecular dosimetry of the Chemical Mutagen Ethylmethanesulfonate (EMS) in *Neurospora crassa* and *Saccharomyces cerevisiae*. *Environ. Mut. 4*:300, 1982.

van Zeeland, A.A., G.R. Mohn, A.T. Natarajan, A.G.A.C. Knaap, A.D. Tates, B.W. Glickman, F.J. de Serres, H.E. Brockman, and C.S. Aaron. Molecular Dosimetry of the Chemical Mutagen Ethyl Methanesulfonate comparison of Mutation Induction in *Escherichia coli*, *Neurospora crassa*, V79 Chinese Hamster Cells, L5178Y Mouse Lymphoma Cells and Some *in vitro* and *in vivo* Cytological Results. *Mutation Research 74*:184, 1980.

Zeiger, E. Knowledge Gained from the Testing of Large Numbers of Chemicals in a Multi-Laboratory, Multi-System Mutagenicity Testing Program. In: *Environmental Mutagens and Carcinogens (Proc. of the 3rd International Conf. on Environmental Mutagens)*, T. Sugimura, S. Kondo, and H. Takebe, (EDS) University of Tokyo Press, Tokyo/Alan R. Liss, Inc., New York, 337–344, 1982.

Zeiger, E. An Overview of Genetic Toxicity Testing in the National Toxicology Program. In: *NY Acad. Sci. 407*:387–394, 1983.

Zeiger, E., S.R. Haworth, W.T. Speck, K. Mortelmans. Phthalate Ester Testing in the NTP Environmental Mutagenesis Test Development Program, *Environ. Health Perspect. 45*:99–101, 1982.

Zeiger, E., D.A. Pagano. Comparative Mutagenicity of Dimethylaminozobenzene and Analogues in Salmonella. *Carcincogenesis 3*:559–561, 1982.

Zeiger, E., D.A. Pagano, A.A. Nomeir. Structure/Activity Studies on the Mutagenicity of Tris (2,3-Dibromopropyl) Phosphate (Tris-BP) and its Metabolites in Salmonella, Environ. *Mutagen 4*:271–277, 1982.

Zeiger, E., M.D. Shelby, K.J. Risko, and B.H. Margolin. Salmonella, Mutagenicity, Collaborative Study, Laboratory Variability, Genetic Drift (Abstract), *Mutat Res.* (European Environmental Mutagen Society Annual Meeting-Helsinki, Finland 6-24-82).

Zimmerman, F.K., F.J. de Serres, and M.D. Shelby. Meeting Report: Workshop on Systems to Detect Induction of Aneuploidy by Environmental Mutagens. *Mutation Res:64*: 279–285, 1979.

Development of Test Systems to Correlate Carcinogenic and Mutagenic Activity

Brockman, H.E. and F.J. de Serres. Short-term Tests for Genetic toxicity. In: Short-term Tests for Environmentally Induced Chronic Health Effects (EDS), Arvil D. Woodhead and Michael D. Waters, Office of R & D, U.S. Environmental Protection Agency, 11–38, 1983.

Brooks, Peter and F.J. de Serres. Overview of Assay System Performances. In: Evaluation of Short-term Tests for Carcinogens: Report of the International Collaborative Program, Elsevier/North Holland, Amsterdam, 96–111, 1981.

Drake, J.W. and F.J. de Serres (Rapporteurs), W.J. Darby, V.C. Dunkel, D.G. Longfellow, H. Ott, I.F.H. Purchase, C. Ramel, C. Schlatter, and E.M.B. Smith. Report 11. Rationale for Deployment of Short-term Assays for Evidence of Carcinogenity—Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal, IARC Monographs, Supplement 2, Reports of IARC *ad hoc* Working Groups, Hannover, FRG, June 7–9, 1979, 295–308, 1980.

Loprieno, N. and R.C. von Boprstel (Rapporteurs), L. Herrera and F.J. de Serres. Report 4. Mutagenesis Assays with Yeasts and Molds—Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal, IARC Monographs, Supplement 2, Reports IARC *ad hoc* Working Groups, Hannover, FRG, June 7-9, 1979, 135-155, 1980.

Purchase, I.F.H., and F.J. de Serres. International Programme for the Evaluation of Short-term Tests for Carcinogenicity. *Arch. Toxicol.* *46*: 195-198, 1980.

de Serres, F.J. Assessment of the International Programme for the Evaluation of Short-term Tests for Carcinogenicity, Quo Vadis? - Tests Courts de Cancerogenes/Short-term Tests for Carcinogenesis, Montpellier, February 4-5, 1981. (EDS) S. Garattini, G. Mazue, R. Roncucci, and G.M. Williams, Excerpta Medica, Amsterdam-Oxford-Princeton, 327-339, 1983.

de Serres, F.J. Evaluation of Short-term Tests for Mutagenicity in the International Program for Chemical Safety. In: Environmental Mutagens and Carcinogens. (Proceedings of the 3rd International Conference on Environmental Mutagens). T. Sugimura, S. Kondo, and H. Takebe (EDS). Published jointly by the University of Tokyo Press, Tokyo/Alan R. Liss, Inc., New York, 747-752, 1981.

de Serres, F.J. The International Program for the Evaluation of Short-term Tests for Carcinogenicity (IPESTTC), presented at the workshop "Short-term Bioassays in the Analysis of Complex Environmental Mixtures II." (EDS) M.D. Waters, S.S. Sandhu, J.L. Huisingh, L. Claxton, and S. Nesnow, Plenum Press, New York, 485-506, 1981.

de Serres, F.J. The International Program for the Evaluation of Short-term Tests for Carcinogenicity (IPESTTC), presented at the workshop "The Predictive Value of *in vitro* Short-term Screening Tests in the Evaluation of Carcinogenicity," April 26-28, 1980, Dalen, The Netherlands.

de Serres, F.J. and J. Ashby. (EDS), Evaluation of Short-term Tests for Carcinogens: Report of the International Collaborative Program, Elsevier/North-Holland, Amsterdam, 1981.

de Serres, F.J. and J. Ashby. Preface in: Evaluation of Short-term Tests for Carcinogens: Report of the International Collaborative Program, Elsevier/North-Holland, Amsterdam, xi-xiii, 1981.

de Serres, F.J., G.R. Hoffman, J. von Borstel, R. Jagannath, N. Loprieno, J. Parry, D. Sharp, I.A. Zakharov, and F. Zimmerman. Summary Report on the Performance of Yeast Assays. In: Evaluation of Short-term Tests for Carcinogens: Report of the International Collaborative Program, F.J. de Serres and J. Ashby (EDS) Elsevier/North Holland, Amsterdam, 67-76, 1981.

de Serres, F.J. and M. Shelby. The Salmonella Mutagenicity Assay: Recommendations, Meeting held in Arlington, VA, August 28-30, 1978, Science 203:563-565, 1979.

Shelby, M.D. International Program for the Evaluation of Short-term Tests for Carcinogenicity (Abstract), Chem. Abstracts 98:218, 1983.

Shelby, M.D., T. Matsushima. Mutagens and Carcinogens in the Diet and Digestive Tract. *Mutat. Rs.* *85*:177-183, 1981.

Studies To Determine How To Extrapolate Data From Experimental Mutagenicity Tests To Man

Ansari, A.A. Separation of Rat Lactate Dehydrogenase Isoenzyme C_4 from Other Isoenzymes by Affinity and Ion-exchange Chromatography. *Biochem. J.*, *199*:75-79, 1981.

Ansari, A.A., M.A. Baig, and H.V. Malling. *In vivo* Germinal Mutation Detection with 'Monspecific' Antibody Against Lactate Dehydrogenase-x. *Proc. Natl. Acad. Sci (USA)*, *77*: 7352-7356, 1980.

Burkhart, J.G., J. Benziger, K. Svensson and H.V. Malling. An Evaluation of Heterologous Antibodies to Lactate Dehydrogenase-C in the Detection of Mutations. *Mutation Res.* *148*:135-149, 1985.

Burkhart, J.G., A.A. Ansari, and H.V. Malling. Localization of Cytoplasmic Lactate Dehydrogenase-X in Spermtozoa. *Archives of Andrology* 9:115-120, 1982.

Burkhart, J.G., C.P. Ray, and H.V. Malling. Effect of Procarbazine Treatment of Mice on a-glycerolphosphate Dehydrogenase Activity and Frequency of Selected Abnormalities in Sperm. *Mutation Res.* *92*:249-256, 1982.

Burkhart, J.G. and H.V. Malling. Sperm Abnormalities in the PL/J Mouse Strain: A Description and Proposed Mechanism for Malformation. *Gamete Research* *4*:171-183, 1981.

de Serres, F.J. Aneuploidy as a Human Health Problem of Significance in Environmental Mutagenesis. *Environ. Health Perspect.* *31*:1-2 (1979).

Wyrobek, A.J., L.H. Gordon, J.G. Burkhart, M.C. Francis, R.W. Kapp, G. Letz, H.V. Malling, J.C. Topham, and M.D. Whorton. An Evaluation of the Sperm Morphology Test and Other Sperm Tests in Nonhuman Mammals. *Mutation Res.*, *115*:1-72, 1983.

Development of Methodology for Monitoring the Human Population for Changes in the Genetic Load

Brusick, D., F.J. de Serres, R.B. Everson, M.L. Mendelsohn, J.V. Neel, M.D. Shelby, and M.D. Waters. Monitoring the Human Population for Mutagenic Effects: Detection of Gene Mutations and DNA Damage. In Bloom, A.D. (ED): *Guideline for Studies of Human Populations Exposed to Mutagenic and Reproductive Hazards*. March of Dimes Birth Defects Foundation, 1981, 111-140.

de Serres, F.J. Some Aspects of Chemical Mutagenesis and Human Population Monitoring—An Overview. In: Chemical Mutagenesis, Human Population Monitoring and Genetic Risk Assessment. K.C. Bora, G.R. Douglas, and E.R. Nestmann (EDS), Progress in *Mutation Research*, Vol. 3, Elsevier Biomedical Press, Amsterdam, 107-110, 1982.

Wilcox, A.J. Surveillance of Pregnancy Loss in Human Populations. *Am. J. Industr. Med.* 4:285–291, 1983. (Also in Mattison, D.R. (Ed.), *Reproductive Toxicology*. New York, Alan R. Liss, 1983.)

Wilcox, A.J. and B.C. Gladen. Spontaneous Abortion: The Role of Heterogeneous Risk and Selective Fertility. *Early Human Development* 7:165–178, 1982.

Development of Methodology for Epidemiological Investigations

Brown, S.M. The Use of Epidemiologic Data in the Assessment of Cancer Risk. *J. Environ. Pathol. Toxicol. 4(2,3)*:573–580, 1980.

Everson, R.B. Individuals Transplacentally Exposed to Maternal Smoking May be at Increased Cancer Risk in Adult Life. *The Lancet*, July 19, 1980, 123–127.

Everson, R.B., N.M. Gad-el-Mawla, M.A.M. Attia, E.M. Chevlen, S.S. Thorgeirsson, L.A. Alexander, P.M. Flack, N. Staiano, and J.L.. Zeigler. Analysis of Human Urine for Mutagens Associated with Carcinoma of the Bilharzial Bladder by the Ames Salmonella Plate Assay: Interpretation Employing Quantitation of Viable Lawn Bacteria. *Cancer 51*:371–377, 1983.

Hogan, M.D., and D.G. Hoel. Estimated Cancer Risk Associated with Occupational Asbestos Exposure. *J. Risk Anal. 1(1)*:67–76, 1981.

Milne, K.L., D.P. Sandler, and R.B. Everson. Lung Cancer and Occupation in Alameda County: A Death Certificate Case-Control Study. *Am. J. Indut. Med.* 4:565–575, 1983.

Rogan, W.J. Analytical Chemistry Needs for Environmental Epidemiology. In, McKinney, J. (Ed.): *Environmental Health Chemistry*. Ann Arbor, Ann Arbor Science Publishers, 1980, 123–34.

Rogan, W.J. The Sources and Route of Childhood Chemical Exposures. *J. Pediatr.* 97:861–865, 1980.

Wilcox, A.J. Surveillance of Pregnancy Loss in Human Populations. *Amer. J. Ind. Med.*, Journal Symposium on Reproductive Toxicology, D.R. Mattison, Ed. (in press).

Wilcox, A.J., and B.C. Gladen. Spontaneous Abortion: The Role of Heterogeneous Risk and Selective Fertility. *Early Human Development* (in press).

Wilcox, A.J., A.E. Treloar, and D.P. Sandler Spontaneous Abortion Over Time: Comparing Occurrence in Two Cohorts a Generation Apart. *Amer. J. Epidemiol. 114*:548–553, 1981.

Joint Scientific Conferences and Workshops

ENVIRONMENTAL MUTAGENESIS AND CARCINOGENESIS PANELS

Date: September 4-6, 1980

Place: Karuizawa, Japan

Summary: The Ninth Joint Panel Conference was held on the subject of "Mutagens and Carcinogens in the Diet and Digestive Tract." Nineteen papers were reported in five sessions of 1) mutagens and carcinogens in raw foods, 2) mutagens and carcinogens produced in cooking and processing, 3) modulation of mutagenic and carcinogenic activity, 4) mutagens and carcinogens found in the digestive tract, and 5) mutagens and carcinogens in human and animal waste. Current information about mutagens/carcinogens produced during the cooking of foods was reported by Japanese and U.S. scientists. Close collaboration between the two groups on this subject evolved.

The summary report of the meeting was published in *Mutation Research 85*:177-183 (1981).

Date: November 6, 1981

Place: Livermore, California

Summary: The Third Joint Workshop on the Current Status of Research on Food Mutagens was held on November 6, 1981, at Lawrence Livermore National Laboratory (LLNL). The workshop was attended by three Japanese scientists, seven LLNL staff members directly involved in this research as well as other LLNL Biomedical Science Division staff. The emphasis was primarily on a discussion of the problem of generation of mutagens with high-temperature cooking. The fractionation and identification of mutagenic components as well as strategies to perform studies on these isolated components to evaluate their mutagenicity in a wide range of experimental organisms and their carcinogenicity in rodents were also discussed. The critical aspect of the discussion was the need to develop experimental approaches to measure exposure to somatic and germ cells and to evaluate the human health consequences in terms of cancer and genetic disease.

Date: February 7-9, 1983

Place: Honolulu, Hawaii

Summary: The Tenth Joint Panel Conference was held in Honolulu (instead of the originally planned meeting place in Japan) and 8 Japanese scientists and 14 U.S. scientists participated. Under the subject of "Population Monitoring: Methods and Applications," 16 reports were presented and discussed in the 5 sessions on 1) evaluation of reproductive effects and spontaneous abortions, 2) evaluation of chromosome damage, 3) urine and semen analysis, 4) population monitoring—new methods and surveillance, and 5) population monitoring and future directions.

The meeting reports were published in *Mutation Research, 130*:73-77 (1984).

Date: February 6-8, 1984

Place: Kailua-Kona, Hawaii

Summary: The 11th Joint Conference on Test Method Development, Validation, and Utilization in Environmental Mutagenesis and Carcinogenesis was held in Kailua-Kona, Hawaii, February 6-8, 1984. The conference was attended by 10 scientists from Japan and 15 scientists from the United States. Test method development was discussed in terms of improvements in the rodent bioassays for cancer in the National Toxicology Program, improved methods for qualitative and quantitative evaluation of clastogenicity of carcinogens in the National Institute of Hygienic Sciences in Japan, and better measurement of the induction of chromosomal damage in exposed individuals by assaying for micronuclei in circulating erythrocytes. Test method validation included a discussion of an International Collaborative Study to evaluate short-term *in vitro* tests for carcinogenicity. A total of 10 chemicals were tested to determine whether eukaryotic assays can detect chemical carcinogens that are inactive or cannot be readily detected with the Salmonella assay. In addition, a survey was presented of research in the National Cancer Center Research Institute, Tokyo on short-term tests for mutagenicity and their correlation with long-term rodent bioassays for cancer. A similar survey of chemicals tested in short-term assays for mutagenicity and long-term cancer bioassays for cancer in the National Toxicology Program was also presented. Discussions in the session on test method utilization centered on attempts to determine the correlation between carcinogenic and mutagenic activity and the problems imposed in this analysis by a too limited data base, particularly that on noncarcinogens. Papers were also presented on new National Toxicology Program data to evaluate the potential of chemicals to induce genetic damage in mammalian germ cells. Research in Japanese studies (National Cancer Center Research Institute, Tokyo) and United States studies (Lawrence Livermore National Laboratory) on mutagens and carcinogens in food were also contrasted. Work in the latter case is primarily focused on the effects of high-temperature cooking, whereas the Japanese work includes work of this type as well as broad surveys to evaluate common processed foods and beverages as well as edible plants. One of the most interesting data bases presented was that on bacterial assays on 1,026 new chemical substances evaluated at the Institute of Medical Sciences at the University of Tokyo. The chemicals include raw materials and intermediates of industrial processes. By January of 1984 a total of 132 (12.8%) were shown to be mutagenic in Salmonella and/or *E. coli*. Tests carried out on 151 existing chemical substances showed that 46 (30%) gave a positive bacterial test. It was apparent from the papers presented during the course of this conference that highly relevant work is being performed in each of the three activity areas, and that data bases are being developed in both the United States and Japan that will permit a comprehensive evaluation of the general utility of the short-term test technology. A meeting report was published in *Mutation Research 130*:353–359, 1984.

Date: December 19, 1984

Place: Honolulu, Hawaii

Summary: The Fourth Joint Workshop to evaluate research priorities in the area of food mutagen research was held in Honolulu, Hawaii on December 19, 1984, immediately following a 2-day symposium on the same topic held at the 1984 International Chemical Congress of Pacific Basin Societies, December 17–18. Because of the extremely rapid developments in the field, a rapidly expanding data base, and increased need for resources, the workshop provided an opportunity to identify and prioritize research needs. The workshop was attended by 9 scientists from Japan, 10 from the United States, and 2 from Canada and Australia.

The meeting report on the symposium and workshop will be published in *Environmental Health Perspectives*.

Date: January 8–10, 1985

Place: Shimoda, Japan

Summary: The Twelfth Joint Panel Conference on "Mutagenesis and Carcinogenesis by Nitropyrenes and Cancer Chemotherapeutics" was attended by 25 Japanese scientists and 8 U.S. scientists. Sixteen papers were presented in the 4 sessions of 1) mutagenicity of nitropyrenes, 2) carcinogenicity of nitropyrenes, 3) secondary cancer in chemotherapy patients, and 4) genetic toxicology studies in cancer chemotherapy.

The summary report of this meeting will be published in *Mutation Research*.

HEPATITIS PANEL

Drs. Toshitsugu Oda and Saul Krugman, Chairmen, respectively, of the Hepatitis Panel, U.S.-Japan Cooperative Medical Science Program

Dr. Toshitsugu Oda, Chairman (1979–), Director
National Medical Center
1-21-1, Toyama, Shinjuku-ku
Tokyo 162, Japan

Dr. Saul Krugman, Chairman (1979–), Professor
New York University Medical Center School of Medicine
Department of Pediatrics
550 First Avenue
New York, New York 10016

PANEL MEMBERS

Japan

Dr. Tyoku Matsuhashi (1979–)
Director
Department of Applied Immunology
National Institute of Health
2-10-35, Kamiosaki, Shinagawa-ku
Tokyo 141, Japan

Dr. Kusuya Nishioka (1979–)
Vice-Director
Tokyo Metropolitan Institute of Medical Science
3-18-22, Hon-Komagome, Bunkyo-ku
Tokyo 113, Japan

Dr. Kazuo Ohkochi (1979–)
Professor
Department of Central Laboratory
Faculty of Medicine
Kyushu University
3-1-1, Maide, Higashi-ku
Fukuoka 812, Japan

United States

Dr. Thomas C. Merigan (1979–)
Professor
Department of Medicine
Stanford University Medical School
Stanford, California 94305

Dr. Robert H. Purcell (1979–)
Head, Hepatitis Virus Section
Laboratory of Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 7, Room 301
Bethesda, Maryland 20205

Dr. Allan G. Redeker (1979–)
Professor of Medicine
University of Southern California
Liver Unit, Rancho Los Amigos
7601 East Imperial Highway
1100 Building, Room 53
Downey, California 90242

Dr. Toshio Shikata (1979-)
Professor
Department of Pathology
Nihon University School of Medicine
30-1, Oyaguchi-Kamimachi, Itabashi-ku
Tokyo 173, Japan

Dr. Cladd E. Stevens (1984-)
Laboratory of Epidemiology
The New York Blood Center
310 East 67th Street
New York, New York 10021

Former Member

Dr. Wolf Szmuness (1979-82, Deceased)
Head, Laboratory of Epidemiology
The New York Blood Center
310 East 67th Street
New York, New York 10021

Japanese Hepatitis Panel

BROAD GOALS

Viral hepatitis is a serious health problem in many areas of the world including Japan, the United States, and Asia. The two types most commonly recognized are types A (HAV) and B (HBV). A third type of viral hepatitis has been identified and is referred to as non-A, non-B hepatitis (NANB). Two or more distinct agents of blood-borne NANB are thought to exist. In addition, existence of epidemic non-A, non-B hepatitis transmitted by fecal-oral routes was first recognized during the past 5 years. Another new type, delta hepatitis virus (HDV) which requires coinfection with HBV, was recognized.

The long-term objective of the program is to define conditions for the control of viral hepatitis by improved environmental conditions, therapy, or prophylaxis. During the past decade, a hepatitis B virus vaccine has been developed, and practical methods to prevent the hepatitis B virus carrier state are available.

Research areas of high interest to the program are:

1. Biochemical and biophysical characterization of hepatitis agents including that of non-A, non-B hepatitis.
2. Seroepidemiologic studies in Japan, the United States, and Asian countries to identify prevalence and incidence of infections caused by various hepatitis viruses.
3. Development and evaluation of vaccines and antiviral compounds for prevention and treatment of disease with particular emphasis on therapeutic measures for chronic carriers of type B virus. Implicit in these objectives is underlying appropriate work on *in vitro* cultivation of hepatitis agents.
4. Long-term studies to determine the incidence of hepatoma as a sequela of chronic viral hepatitis infection.

PROGRESS IN PRIORITY AREAS

The most important accomplishments were the development of the hepatitis B vaccine (HB vaccine) and high titer human immunoglobulin (HBIG) against hepatitis virus infection.

The method for producing HB vaccine from human plasma was established in Japan. Japanese vaccines prepared in two institutions were licensed and will be available commercially in 1985. Both of the Japanese vaccines meet the current WHO requirements set up in 1983. Studies have shown that more than 95% of perinatal transmission of HBV from HBe antigen positive mothers to their infants can be prevented by immunoprophylaxis employing the combined use of HBIG and HB vaccine. This means offers an approach to control the route of transmission of HBV which has lead to the production of more than 200 million HBV carriers in the world and which has been linked to more than 80% of all cases of primary hepatocellular carcinoma (PHC). These studies are the first to demonstrate the possibility of prevention of one form of cancer by immunization; furthermore, this approach promises to cut the origin of hepatitis B virus reservoirs which are linked to chronic liver diseases and which cause parenteral infection in other persons.

To achieve these results, members of the panel and their associates have been making important contributions in their own fields of research and have been collaborating extensively with members in the United States and collaborators in other Asian countries.

At present, the main projects which are under extensive study are the development of better HBV vaccines, antiviral treatment of HBV carrier state, development of hepatitis A vaccine, and further characterization of non-A, non-B hepatitis viruses.

Before starting the U.S.-Japan Cooperative Medical Science Program on hepatitis, a U.S.-Japan hepatitis symposium was held in Tokyo, July 17–18, 1978. The first joint meeting of the panel was held in New York, March 24–25, 1980; the second in Hakone, November 20-21, 1980; the third in Washington, D.C., March 22-23, 1982; the fourth in Kyoto, March 9–10, 1983; and the fifth in San Francisco, March 8–10, 1984. The sixth meeting was held in Oiso, March 11-12, 1985.

HBV DNAs of subtypes adr and adw were cloned in *E. coli.*, and their complete nucleotide sequences were determined. The sequence of the viral genome of the adr clone was 3188 nucleotides long, and that of the adw clone was 3200 nucleotides. The adr and adw clones differed from the reported ayw HBV-DNA clone (3182 nucleotides long) in 11.2% and 10.0% of nucleotides, respectively. Heterogeneity of the HBV genome in the clones with the same subtype (adr) was observed. Subtypes adr and adw are found most frequently in Asian countries.

The receptor for polymerized human and chimpanzee albumin was identified as a 31-k Da HBs antigen polypeptide. The polypeptide (p31) was composed of a major polypeptide of 8-k Da daltons (p8) that contained carbohydrate. P8 could bind to polymerized human or chimpanzee albumin but not to any polymerized albumins from animals non-susceptible to HBV. The amino acid composition of p8 closely resembled that of the 55 amino acid sequence coded by the pre-S region in HBV DNA. Using the monoclonal antibody against p8, a solid-phase RIA was developed for HBs antigen bearing the receptor for polymerized albumin. Since the receptor is thought to be involved in the first step of HBV infection of susceptible liver cells through polymerized albumin, the characterization of p8 is essential to elucidate the mechanism of HBV infection and may contribute to further development of a more effective vaccine.

As for the characterization of non-A, non-B hepatitis agent, it has been shown that non-A, non-B hepatitis virus in the serum which induced characteristic tubular structures in the hepatocyte cytoplasm of chimpanzee was inactivated by mild treatment with β-propiolactone (0.05%, 4°C-20 minutes). The results showed that β-propiolactone is extremely efficacious for the cold sterilization of this type of virus and has practical application.

As for seroepidemiological studies, Asian countries have a higher incidence of perinatal transmission from HBV carrier mothers to their babies. This is considered to be the predominant mode of HBV transmission that leads to the carrier state. The chronic carrier constitutes a high risk group for developing hepatocellular carcinoma. Elucidation of the mode and magnitude of perinatal transmission in each region is essential for the development of prevention programs of HBV infection. The data from Burma, China, Hong Kong, Japan, the Philippines, Singapore, Taiwan, and Thailand were reviewed by Nishioka.

Extensive studies on *in vitro* cultivation and purification of hepatitis A virus have been underway as part of a program to develop an HAV vaccine.

In vaccine development, pullulan is suggested as a possible carrier for the HBs antigen, since it (pullulan) is not antigenic, does not induce blastogenesis, and stimulates IgM and IgG antibody production. It is noteworthy that this compound has poor stimulating activity for IgE and delayed type hypersensitivity responses which are considered to be untoward side effects.

As for HB vaccines and HBIG (HB immunoglobulin), the main focus of attention in Japan is prevention of perinatal transmission from HBe antigen positive carrier mothers to infants. In one study, three injections of 10 mg HB vaccine following repeated intramuscular injections of HBIG (200 I.U.) were given to 881 babies born to HBe antigen positive mothers. Only 33 (3.7%) of the babies became HBV carriers; 96.3% remained free of HBV virus. In another study group, intravenous injection of Fc free HBIG (200 I.U.) was given to 1,100 babies immediately after birth followed by repeated intramuscular injections of HBIg (200 I.U.) and HB vaccine. In the first 6 months, 18 cases (1.7%) became HBV carriers, before the whole schedule of the passive and active immunization had been completed. However, all 375 cases completing the whole schedule were protected from HBV infection. Six patients were found to be nonresponders to HB vaccine after 1 year's followup. The data showed that combined passive and active immunization are extremely efficacious (protective rate in the first cases, 95.8%, and in the latter cases 98.1%) after 1 year's followup. Nevertheless, problems requiring further investigation are nonresponders to HBs vaccine and the babies who turned HBs antigen positive before completion of immunization.

Clinical trials of antiviral agents for treatment of chronic type B hepatitis have included such drugs as interferon (IFN), IFN-α -β, adenine arabinoside (Ara-A) and its monophosphate (Ara-AMP). These studies have been under the sponsorship of the Ministry of Health and Welfare in Japan. These antiviral drugs in almost all cases caused a decrease in HBs antigen, HBV-DNA, and DNA-polymerase levels in the blood. However, after stopping the drugs, the levels returned to the pretreatment levels. Of the 55 chronic carriers who were also HBe antigen positive, 16 (29.1%) developed anti-HBe and 7 (14.5%) developed anti-HBs.

Followup study of patients showed that four cases (2.9%) became seronegative for HBe antigen, and three cases (2.1%) were converted to anti-HBe positive. These results do not satisfy our initial expectation. Further investigations are required to determine the role of these antiviral agents. Furthermore, combination therapy of these individual antiviral agents together with immunostimulants, such as levamisol or cianidasol, should be carried out.

As for studies on primary hepatocellular carcinoma (PHC) as sequelae of chronic viral hepatitis, an additional marker of chronic HBV infection, integration of HBV DNA into human hepatoma cell line as well as in human PHC tissues has been done.

As for the direct evidence of the etiological relationship of persistent HBV infection for PHC, seroepidemiological and clinical followup studies carried out so far in Asian countries were summarized. These studies have shown that more than 80% of PHC in Asian countries were attributable to HBV. HBV-persistent infection precedes the appearance of PHC. Perinatal transmission from HBe antigen-positive mothers to infants at the time of birth is the predominant mechanism for establishment of persistent HBV infection. Based on these clinical and epidemiological findings, prevention studies on perinatal infection of HBV from mother to child were carried out extensively in various Asian countries. Since the highest risk group for development of PHC was identified as the HBs antigen positive group, elimination of the risk factor became available through immunoprophylaxis as described above, and the reduction of PHC cases can be predicted by converting the high risk group into a low risk one.

Collaborative efforts of the U.S.-Japan Hepatitis Program have been performed by exchanging scientists, new information, and materials for research purposes.

In addition to this, since hepatitis and hepatoma are serious health problems in almost all Asian countries, as were mentioned in the guidelines, scientists from China, the Philippines, Indonesia, and Nepal have visited and studied in the laboratories of panel members. They were supported by the World Health Organization, Viral Hepatitis Research Foundation of Japan, Japan Science Promotion Foundation, and the Tokyo Metropolitan Government Exchange Program. The scientists, Drs. Muller, Tao, Feng, and Zhao from Peking Medical College, stayed in Drs. Nishioka and Mayumi's laboratory. Included also were Drs. Wang, Ho Hwan, Zhai, and Jian from Peking Second and First Infectious Diseases Hospitals; Drs. Domingo, Lingao, Lao, and Dy from the University of the Philippines; and investigators from Indonesia, all of whom worked with Drs. Oda, Nishioka, Suzuki, and Mayumi. Through collaboration and exchanging new information among Asian countries, much progress has been made in seroepidemiologic studies and prevention programs.

ACHIEVEMENT OF HEPATITIS PANEL DURING THE PAST FIVE YEARS

Two decades ago, about half of the patients who received blood transfusions suffered from post-transfusion hepatitis. Fear of yellow-blood was a serious problem and became a widespread social problem throughout Japan. In 1952, Professors Amano and Katsunuma reported two ceses of fulminant hepatitis following blood transfusion. And a U.S. ambassador to Japan was no exception to those who suffered such an aftermath of blood transfusion.

No reliable laboratory method was available to detect the etiological agents of viral hepatitis, and the hepatitis virus problem was considered one of the most difficult problems of microbiology. In the field of liver cancer, attention had been focused on chemical carcinogenesis in experimental animals and little progress had been made in understanding human primary liver cancer.

Considering the magnitude of the health problem, administrative authorities decided to switch from paid donors to a volunteer blood donation system. Although the situation showed much improvement, it was far from totally effective in reducing the incidence of post-transfusion hepatitis.

At that time, Dr. Okochi of the University of Tokyo Hospital found a peculiar antigen in the blood closely linked to post-transfusion. This factor was further identified as Blumberg's Australia antigen, the significance of which was unknown at that time. Later this factor was identified as the surface antigen of HBV, and this cooperative study between two researchers in Japan and the United States became a real breakthrough in the study of hepatitis viruses today.

The principle to avoid the use of Australia antigen positive blood for blood transfusions was first adopted at the University of Tokyo Hospital. This was the real starting point of the program to eradicate all cases of post-transfusion hepatitis B in blood transfusions in both the United States and Japan.

On the other hand, Krugman of New York University kept two serum samples, MS-1 and MS-2, as prototypes of hepatitis materials. These lead to discoveries of both hepatitis viruses A and B.

In Japan, serological methods to improve the sensitivity and specificity for detection of HBs antigen were established. These led to the discovery that more than 80% of primary liver cancer can be attributed to persistent HBV infection, and to the discovery of familial clustering of chronic liver disease and liver cancer. These studies elucidated the perinatal HBV transmission from carrier mothers to their infants, which is considered to be the most predominant route for persistent HBV infection.

With this information available, the U.S.-Japan Cooperative Research Program on Hepatitis was formulated in 1979, and further collaboration of researchers in other countries, including Asian nations, was started. Since then, results in the research fields as described above have been obtained.

Development in immunological test reagents made possible the diagnosis of hepatitis A and B and the monitoring of HBV markers, the latter of which led to the development of a strategy to control post-transfusion type B hepatitis and hepatitis B infection in medical institutions. Development of HBIG and HB vaccine set up the immunoprophylaxis measures, both passive and active immunization, against HBV infection. When these measures were applied to prevention of perinatal infection, the preventive efficacy rate showed almost 100%, and they were evaluated as the most effective and safest vaccines ever used in anti-microbial prevention.

These results contributed vastly to improvement in practical medical care as well as to general health care administration. The Ministry of Health and Welfare of Japan proposed the immunoprophylaxis of all babies born to HBe antigen-positive mothers to prevent the persistent HBV infection. This was evaluated by WHO and UICC as the most effective system to prevent one type of human neoplasm, primary liver cancer which is prevalent in Asian countries.

At present, the following items are under extensive study: improvement of HBV vaccines; development of HAV vaccine; characterization of etiological agents of non-A, non-B hepatitis; further analysis of hepatocarcinogenesis; and further studies of the activity of antiviral compounds against hepatitis virus.

FUTURE GOALS

In the past two decades, remarkable progress in hepatitis B control has been achieved. Since the magnitude of hepatitis and liver cancer has been elucidated in both Japan and the United States as well as in Asian countries, the studies of hepatitis are not focused on the mere control of one single communicable disease, but are also deeply concerned with control of a specific cancer.

Based on the accumulated results at this time, the following items are considered to be high priority items for future study:

1. To develop second, third, and fourth generation HB vaccines with the goal of control of hepatitis B virus infections and primary liver cancer.
2. To develop antiviral therapy with the goal of prevention of chronic sequelae of HBV infection (carrier state, chronic hepatitis, liver cirrhosis, and primary liver cancer).
3. To elucidate the nature of blood-borne non-A, non-B hepatitis, the most prevalent forms of posttransfusion hepatitis in both countries at present.
4. To develop hepatitis A vaccine together with elucidation of the nature of water-borne type non-A, non-B hepatitis prevalent in Southeast Asian countries.

GUIDELINES

1. Seroepidemiological studies of viral hepatitis in Japan and various Asian countries.
2. Long-term studies of chronic viral hepatitis infection, with special reference to HBe seroconversion.
3. Anti-viral compounds for the treatment of chronic active hepatitis, type B.
4. Development and evaluation of HB vaccine and immunoglobulin for prevention.
5. Biochemical and biophysical characterization of hepatitis viruses, including non-A, non-B hepatitis virus.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
Toshitsugu Oda National Medical Center, Japan	Prevention of hepatitis B virus infection Sequele of viral hepatitis
Kusuya Nishioka Tokyo Metropolitan Institute of Medical Science	Characterization of HB virus and recombinant HBsAg Seroepidemiology of type B hepatitis in Asian countries
Toshio Shikata Nihon University School of Medicine	Experimental studies on non-A, non-B virus infection Pathology of viral hepatitis
Kazuo Okochi Kyushu University Faculty of Medicine	Surveillance of post-transfusion hepatitis
Tyoku Matuhasi National Institute of Health, Japan	Hepatitis B immunoglobulin and its use for prophylaxis
Nakao Ishida Tohoku University	Non-A, non-B virus marker Gamma-interferon inducer
Hiroshi Oka Tokyo University	Fulminant hepatitis and its treatment Hepatocyte regeneration
Akira Ohbayashi Tokyo National Chest Hospital	Treatment of chronic hepatitis due to HB virus
Shiro Iino Tokyo University	Treatment of HB virus carrier
Hiroshi Yoshizawa Hamamatsu Medical College	Prevention of HB virus infection
Eiichi Tokunaga Japan Red Cross, Blood Center	Production of HB immunoglobulin and α-interferon
Yasuo Moritsugu National Institute of Health, Japan	Propagation of hepatitis A virus
Hiroto Shimojo National Institute of Health, Japan	Hepatitis A virus vaccine production
Makoto Mayumi Jichi Medical College	Non-A, non-B virus markers Characterization of HB virus
Keiji Mitamura Tsukuba University	Integration of HBV DNA to the liver cell DNA
Hiroshi Suzuki Yamanashi Medical College	Treatment of chronic viral hepatitis, with anti-viral agents and immunomodulators
Seiichi Furuta Shinshu University	Epidemiology of acute viral hepatitis, including non-A, non-B
Fumihiro Ichida Niigata University	Pathology of type A hepatitis Treatment of chronic viral hepatitis
Yoshiyata Takahashi Gifu Central Hospital	Fulminant hepatitis-clinical surveillance and treatment

Investigator and Institution	*Title*
Kan Matsushita Hamamatsu Medical College	Epidemiology of viral hepatitis and its sequelae in the whole nation
Nobu Hattori Kanazawa University	Hepatocellular carcinoma: integration of HB virus DNA and its significance
Sukeo Yamamoto Osaka City University	Immunological studies on viral hepatitis
Tatsuo Takino Kyoto Prefectural University of Medicine	Treatment of chronic hepatitis
Hideo Nagashima Okayama University	Immunological studies on the chronicity of viral hepatitis
Michitami Yano National Nagasaki Central Hospital	Prevention of HB virus maternal infection

U.S. Hepatitis Panel

BROAD GOALS

The organization of the Hepatitis Panel as a component of the U.S.-Japan Cooperative Medical Science Program in 1979 was a logical expansion of the joint cooperative research effort. Viral hepatitis, especially hepatitis B, is a major worldwide public health problem. It has had an extraordinary impact on the health and well-being of people of all ages in Asia as well as most countries of the world.

The development of sensitive markers of hepatitis A virus (HAV) and hepatitis B virus (HBV) infection has provided the technology required to conduct worldwide seroepidemiologic surveys. In addition, the use of these markers has revealed a third type of hepatitis proved to be non-A, non-B (NANB) in its etiology. When the causative agents of NANB hepatitis are identified, their nomenclature will be designated. More recently a fourth type of hepatitis has been associated with infection by a unique hepatitis virus, the so-called "delta agent" that requires HBV synthesis for its expression. It has been proposed that this agent be designated hepatitis D virus (HDV).

Hepatitis B is the most important and the most serious type of hepatitis in most countries of the world. Seroepidemiological surveys have revealed that about 5% of the population of the world are asymptomatic HBV carriers. The carrier rate in North America and Western Europe is less than 0.5% but in many Asian and African countries it may exceed 10%. It has been estimated that the number of HBV carriers exceeds 150 million worldwide.

Each year HBV infections occur in about 200,000 persons in the United States. Of these, about 50,000 have clinically recognizable hepatitis with jaundice. About 10,000 may require hospitalization and about 10,000 to 20,000 will become chronic HBV carriers. In addition, about 4,000 persons will die of HBV-related cancer of the liver.

Complete recovery from HBV infection may take 4 months or longer. Chronic liver disease may complicate mild or asymptomatic infection as well as severe hepatitis B infection. The acute disease may become chronic in 5% to 10% of cases; a fatal outcome may occur in 1% to 2%. However, recent reports of combined chronic hepatitis B and delta infection indicate that the mortality rate may exceed 10% under these circumstances. In many Asian and African countries, HBV-related hepatoma is the most common malignant tumor, accounting for 20% to 40% of all cancer deaths. In contrast, hepatoma accounts for only 2% of malignant tumors in the United States.

The long-term objective of the United States-Japan Hepatitis Program is to define conditions for the control of viral hepatitis by improved environmental conditions, therapy, or prophylaxis. In order to achieve these objectives the research areas of high interest to the program are:

1. Biochemical and biophysical characterization of hepatitis agents.
2. Seroepidemiologic studies in Japan, the United States, and Asian countries to identify prevalence and incidence of infections caused by the various hepatitis viruses. Implicit in these objectives is appropriate work on *in vitro* cultivation of hepatitis agents.
3. Development and evaluation of vaccines and antiviral compounds for prevention and treatment of illness with particular emphasis on therapeutic measures for chronic carriers of type B hepatitis virus.
4. Long-term studies to determine the incidence of cirrhosis and hepatocellular carcinoma as sequelae of chronic viral hepatitis.

SUMMARY OF THE FIRST FIVE YEARS

The past 5 years showed continued rapid progress in our understanding and control of the hepatitis viruses. Because these viruses differ markedly one from the other, they are covered individually.

Hepatitis A Virus (HAV)

The widespread availability of diagnostic tests for HAV infection has resulted in a better appreciation of the relative importance of HAV in the etiology of viral hepatitis. This virus accounts for 25%-40% of such disease in the United States but its relative importance is decreasing. HAV has been classified as a picornavirus. Its 7500-base RNA genome was molecularly cloned and sequenced. The development of HAV vaccines is in progress. Preliminary attempts to develop a live attenuated vaccine were promising but further attenuation of candidate strains is necessary before they will be suitable for wider testing. Killed vaccines have been developed and are undergoing preliminary clinical testing.

Hepatitis B Virus (HBV)

HBV was shown to account for approximately 25%-40% of clinical hepatitis in the United States and to be on the increase, despite the licensing of an effective and safe killed vaccine in 1982. The application of molecular technology to the study of this virus has strengthened its association with the etiology of hepatoma. Genetically engineered second-generation vaccines prepared in mammalian cells or yeast cells have been developed and show promise of replacing the plasma-derived vaccines currently available. Hepatitis B virus has been classified as a member of a new family of viruses, the Hepadnaviridae. Four similar viruses have been identified in woodchucks, ground squirrels, domestic ducks, and snakes and several of these have been useful in elucidating the natural history and molecular virology of the hepadnaviruses.

Hepatitis Delta Virus (HDV)

The unique relationship between HDV and HBV has been further studied. The HDV appears to be a unique human pathogen that is defective and requires coinfection with HBV (which serves as a helper virus) for its replication. The 36 nm particle associated with infection was shown to contain a unique antigen, delta antigen, and a small (1.75 Kb) putative genome of RNA. HDV was shown to have a world-wide (but spotty) distribution. An exception may be Asia, where very little evidence for HDV infection has been obtained. HDV was found to be associated with up to 50% of fulminant human hepatitis previously thought to be caused by HBV and to be responsible for epidemics of severe hepatitis among native populations in South America and illicit drug users in the United States.

Non-A, Non-B Hepatitis Virus (NANBV)

NANBV has continued to be a mystery for hepatitis researchers. The failure to detect an antigen-antibody system or to identify a genome unique to this agent (or agents) has limited progress. Two or more distinct agents of blood-borne NANBV are thought to exist, based upon epidemiologic investigations and transmission studies in chimpanzees. The latter studies also permitted the identification of essential lipids in at least some strains of NANBV.

Epidemic Non-A, Non-B Hepatitis Virus (ENANBV)

Existence of this presumed virus was first recognized during the past 5 years. It was shown to exist in southern Asia and probably throughout the Middle East. Its epidemiology was similar to that of HAV—predominantly a water-borne disease with major epidemics separated by endemic disease. Evidence has accumulated that the etiologic agent is a 27 nm virus that may be a second serotype of HAV, but attempts to characterize it further have been frustrated by lack of clinical materials, inability to infect tissue cultures with it, and difficulty in transmitting it to laboratory animals.

PROGRESS IN PRIORITY AREAS

Biochemical and Biophysical Classification of the Hepatitis Agents

Multiple strains of HBV have been molecularly cloned and sequenced and amino acid substitutions unique to the different subtypes identified. Synthetic peptides representing different regions of the capsid proteins are being studied as candidate third-generation vaccines. The DNA polymerase of the virion was shown to have a probable second enzyme activity (reverse transcriptase) and to mediate the synthesis of replicative intermediate forms of the virus containing DNA-RNA hybrid molecules.

Several strains of HAV have been similarly cloned and sequenced and the capsid proteins identified. Amino acid sequences predicted from analysis of nucleotide sequences were not closely related to any recognized genus of picornavirus.

Part of the putative genome of the hepatitis delta virus was cloned and sequenced. Radioactivity labeled cDNA was used as a probe for delta RNA in clinical specimens and was found to be useful for the diagnosis of delta infection in chronic hepatitis.

Experimental hepatitis B vaccine tested in clinical trial with NIAID volunteers, August 1978.

Seroepidemiologic Studies

The application of sensitive diagnostic assays for infection with HAV and HBV to consecutive cases of hepatitis in different regions of the United States revealed significant differences in attack rates in different geographic regions and at different times. The overall approximate relative importance of the hepatitis viruses in the etiology of clinical hepatitis during the past 5 years was HAV—25%; HBV—50%; and NANBV—25%. However, during the past 1–2 years, type A hepatitis has continued to decline and type B hepatitis has continued to increase, resulting in similar incidence figures for the two viruses in 1984.

Vaccines and Antivirals

Plasma-derived hepatitis B vaccine was licensed in the United States in 1982. Second generation vaccines prepared by genetic engineering have been developed and are in early clinical trials. Both killed and live hepatitis A vaccines are being developed. They are in early clinical testing and will probably require additional developmental research before they can be considered for licensing.

Antiviral treatment of viral hepatitis has not yet become an accepted therapy. New antivirals will need to be developed and, possibly, new model systems for their evaluation before practical antiviral therapy will be a reality.

Long-term studies of HBV infection to determine if HB vaccination will prevent hepatoma are in progress in Taiwan and in the planning stages in The Gambia. The woodchuck hepatitis virus animal model (in which the virus also appears to cause hepatoma) is being developed for the purpose of evaluating the role of hepadnaviruses in carcinogenesis and prevention by vaccination. The incubation period to cancer in the woodchuck is only 1–6 years instead of the 10–60 years observed in humans.

FUTURE GOALS

In spite of the extraordinary progress during the past 5 years many important problems still require solution. The long-term objective of the program continues to be the control of all types of viral hepatitis. To achieve this objective a high priority has been assigned to the following areas of research:

1. Identification and characterization of NANB viral agents. Reports of various candidate NANB viruses will require confirmation. Ultimately it will be important to identify blood donors who are carriers of these agents.
2. Seroepidemiological surveys should be updated to identify the changing patterns of HAV, HBV and HDV infections in various countries of the world.
3. Long-term followup observations of the persistence of immunity following immunization with hepatitis B vaccine are essential to determine the need for booster doses. These studies should include the newly developed DNA recombinant and synthetic polypeptide vaccines as well as those derived from plasma.
4. It will be important to support controlled studies to determine the therapeutic effect of antiviral agents for the prevention of chronic hepatitis B infection.

Viral hepatitis continues to be a serious health problem in most countries of the world. It is hoped that advances in hepatitis research during the next 5 years will enhance prospects toward its control.

Woodchuck *(Marmota monax)*: animal model for studying hepatitis B viral infection.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
Francis V. Chisari Scripps Clinic and Research Foundation	Hepatic bioregulatory molecules, hepatitis, and cancer
Elizabeth R. Dickie Institute for Cancer Research	Quantitative analysis of transmission of hepatitis B
Gordon R. Dreesman Southwest Foundation for Biomedical Research	Immune response to synthetic HBsAg peptides
Robert J. Gerety Food and Drug Administration	Holloman Air Force Base chimpanzee breeding colony
John Gerin Georgetown University	Operation of a molecular virology and immunology laboratory
F. Blaine Hollinger Baylor College of Medicine	Synthetic hepatitis B vaccine studies Studies with hepatitis B vaccines
Ronald C. Kennedy Southwest Foundation for Biomedical Research	Idiotypic analysis of the immune response to HBV
William S. Mason Institute for Cancer Research	Hepatitis B-like virus of the domestic duck

Investigator and Institution	*Title*
James E. Maynard Centers for Disease Control	Hepatitis B vaccine in the People's Republic of China
David R. Milich Scripps Clinic and Research Foundation	MHC-control and cell correlates of HBsAg immune response
Darrell L. Peterson Virginia Commonwealth University	Structure of hepatitis B surface antigen proteins
Jere M. Phillips Meloy Laboratories, Inc.	Facility for nonhuman primates for viral hepatitis experiments
William S. Robinson Stanford University School of Medicine	A hepatitis virus-infected ground squirrel colony The viruses of hepatitis B and ground squirrel hepatitis
William J. Rutter University of California	Molecular analysis of hepatitis B virus
Aleem Siddiqui University of Colorado Health Sciences Center	Hepatitis B virus DNA in *kaposi sarcoma*
John J. Sninsky Yeshiva University	Hepatitis B virus and *E. coli* synthesized viral protein
Jesse W. Summers Institute for Cancer Research	Genome structure and replication of hepatitis B virus Replication and pathogenesis of hepatitis B-like viruses
Wolf Szmuness New York Blood Center	Epidemiology of viral hepatitis in hemodialysis centers Studies of the interruption of maternal to infant transmission of hepatitis B
Bud C. Tennant Cornell University	Breeding and experimental facility for woodchucks *(Marmota monax)*
Harold E. Varmus University of California	The molecular biology of hepatitis B-type viruses
Victor M. Villarejos Louisiana State University Medical Center	Epidemiology of hepatitis B antigens and viral hepatitis

BIBLIOGRAPHY

Books

Viral Hepatitis 1981 International Symposium (W. Szmuness, H.J. Alter, J.E. Maynard, Eds.). The Franklin Institute Press, 843 pp., 1982.

Viral Hepatitis and Liver Disease 1984 International Symposium (G.N. Vyas, J.N. Dienstag, J.H. Hoofnagle, Eds.). Grune and Stratton, Inc., 728 pp., 1984.

Articles

Aach, R.D., W. Szmuness, J.W. Mosley, F.B. Hollinger, R.A. Kahn, C.E. Stevens, V.M. Edwards, and J. Werch. Serum alanine aminotransferase of donors in relation to the risk of non-A, non-B hepatitis in recipients. *N. Engl. J. Med. 304*:989–994, 1981.

Albin, C. and W.S. Robinson. Protein kinase activity in hepatitis B virus. *J. Virol. 34*:297–302, 1980.

Alter, H.J., R.H. Purcell, P.V. Holland, D.W. Alling, and D.E. Koziol. The relationship of donor transaminase (ALT) to recipient hepatitis: Impact on blood transfusion services. *J. Am. Med. Assoc. 246*:630–634, 1981.

Alter, H.J., R.J. Gerety, L.A. Smallwood, R.E. Sampliner, E. Tabor, F. Deinhardt, G. Frosner, and G.M. Matanoski. Sporadic non-A, non-B hepatitis: frequency and epidemiology in an urban U.S. population. *J. Infect. Dis. 145*:886–893, 1982.

Andres, L.L., V.K. Sawhney, G.H. Scullard, J.L. Smith, T.C. Merigan, W.S. Robinson, and P.B. Gregory. Dane particle DNA polymerase and HBeAg: Impact on clinical laboratory, and histologic findings in hepatitis B-associated chronic liver disease. *Hepatology 1*:583–585, 1981.

Aoki, N., M.A. Gerber, S.N. Thung, M.L. Chen, J.K. Christman, P.M. Price, C.S. Flordellis, and G. Acs. Ultrastructural studies of fibroblasts transfected with hepatitis B virus DNA. *Hepatology 4*:84–89, 1984.

Beasley, R.P., L.Y. Hwang, G.C. Lee, C.C. Lan, C.H. Roan, F.Y. Huang, and C.L. Chen. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. *Lancet 2*:1099–1102, 1983.

Beasley, R.P. and L.Y. Hwang. Immunogenicity of hepatitis B virus vaccine (Heptavax-B)—response of healthy adults. *West. J. Med. 140*:731–734, 1984.

Beasley, R.P., L.Y. Hwang, C.C. Lin, C.E. Stevens, K.Y. Wang, T.S. Sun, F.J. Hsieh, and W. Szmuness. Hepatitis B immune globulin (HBIG) efficacy in the interruption of perinatal transmission of hepatitis B virus carrier state. Initial report of a randomized double-blind placebo-controlled trial. *Lancet 2*:388–393, 1981.

Beasley, R.P., L.Y. Hwang, C.C. Lin, M.L. Leu, C.E. Stevens, W. Szmuness, and K.P. Chan. Incidence of hepatitis B virus infections in preschool children in Taiwan. *J. Infect. Dis. 146*:198–204, 1982.

Beasley, R.P., L.Y. Hwang, C.C. Lin, and C.S. Chien. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22,707 men in Taiwan. *Lancet 2 (8256)*:1129–1133, 1981.

Bernier, R.H., R. Sampliner, R. Gerety, E. Tabor, F. Hamilton, and N. Nathanson. Hepatitis B infection in households of chronic carriers of hepatitis B surface antigen: factors associated with prevalence of infection. *Am. J. Epidemiol. 116*:199–211, 1982.

Binn, L.N., S.M. Lemon, R.H. Marchwicki, R.R. Redfield, N.L. Gates, and W.H. Bancroft. Primary isolation and serial passage of hepatitis A virus strains in primate cell cultures. *J. Clin. Microbiol. 20*:28–33, 1984.

Blum, H.E., A.T. Haase, J.D. Harris, D. Walker, and G.N. Vyas. Asymmetric replication of hepatitis B virus DNA in human liver: demonstration of cytoplasmic minus-strand DNA by blot analyses and *in situ* hybridization. *Virology 139*:87–96, 1984.

Blum, H.E., L. Stowring, A. Figus, C.K. Montgomery, A.T. Haase, and G.N. Vyas. Detection of hepatitis B virus DNA in hepatocytes, bile duct epithelium and vascular elements by *in situ* hybridization. *Proc. Natl. Acad. Sci. USA 80*:6685–6688, 1983.

Blum, H., A. Haase, and G.N. Vyas. Molecular pathogenesis of hepatitis B virus infection: Simultaneous detection of viral DNA and antigens in parafin-embedded liver sections. *Lancet 1*:771–775, 1984.

Blumberg, B.S., E.D. Lustbader, and P.L. Whitford. Changes in serum iron levels due to infection with hepatitis B virus. *Proc. Natl. Acad. Sci. USA 78*:3222–3224, 1981.

Bond, W.W., M.H. Peterson, M.S. Favero, J.W. Ebert, and J.E. Maynard. Transmission of type B viral hepatitis via eye inoculation of a chimpanzee. *J. Clin. Microbiol. 15*:533–534, 1982.

Bonino, F., B. Hoyer, E. Ford, J.W.-K. Shih, R.H. Purcell, and J.L. Gerin. The δ agent: HBsAg particles with antigen and RNA in the serum of an HBV carrier. *Hepatology 1*:127–131, 1981.

Bonino, F., B. Hoyer, J. Nelson, R. Engle, G. Verme, and J. Gerin. Hepatitis B virus DNA in the sera of HBsAg carriers: a marker of active hepatitis B virus replication in the liver. *Hepatology 1*:386–391, 1981.

Bonino, F., B.H. Hoyer, J.W.-K. Shih, M. Rizzetto, R.H. Purcell, and J.L. Gerin. The delta hepatitis agent: structural and antigenic properties of delta associated particle. *Infection and Immunity 43*:1000–1005, 1984.

Bradley, D.W., J.E. Maynard, H. Popper, J.W. Ebert, E.H. Cook, H.A. Fields, and B.J. Kemler. Persistent non-A, non-B hepatitis in experimentally infected chimpanzees. *J. Infect. Dis. 143*:210–218, 1981.

Bradley, D.W., J.E. Maynard, E.H. Cook, J.W. Ebert, C.R. Gravelle, K.N. Tsiquaya, H. Kessler, A.J. Zuckerman, M.F. Miller, C. Ling, and L.R. Overby. Non-A/non-B hepatitis in experimentally infected chimpanzees: cross-challenge and electron microscopic studies. *J. Med. Virol. 6*:185–201, 1980.

Brotman, B. and A.M. Prince. Occurrence of AUSAB Test Positivity Unrelated to Prior Exposure to Hepatitis B Virus. *J. Infect. Dis. 150*:714–720, 1984.

Burk, K.H., G.A. Cabral, G.R. Dreesman, R.L. Peters, and H.J. Alter. Ultrastructural changes and virus-like particles localized in liver hepatocytes of chimpanzees infected with non-A, non-B hepatitis. *J. Med. Virol. 7*:1–19, 1981.

Burk, K.H., P.E. Oefinger, and G.R. Dreesman. Detection of non-A non-B hepatitis antigen by immunochemical staining. *Proc. Natl. Acad. Sci. USA 81*:3195–3199, 1984.

Burk, K.H., G.R. Dreesman, G.A. Cabral, and R.L. Peters. Long-term sequelae of non-A, non-B hepatitis in experimentally infected chimpanzees. *Hepatology 4*:808–816, 1984.

Canese, M., M. Rizzetto, R. Novara, W.T. London, and R.H. Purcell. Experimental infection of chimpanzees with the HBsAg-associated delta (δ) agent: an ultrastructural study. *J. Med. Virol. 13*:63–72, 1984.

Celis, E., V.R. Zurawski, Jr., and T.W. Chang. Regulation of T-cell function by antibodies: Enhancement of the response of human T-cell clones to hepatitis B surface antigen by antigen-specific monoclonal antibodies. *Proc. Natl. Acad. Sci. USA 81*:6846–6850, 1984.

Chakraborty, P.R., N. Ruiz-Opazo, D. Shouval, and D.A. Shafritz. Identification of integrated hepatitis B virus DNA and expression of viral RNA in an HBsAg-producing human hepatocellular carcinoma cell line. *Nature 286*:531–533, 1980.

Chang, T.W., E. Celis, R.W. Miller, V.R. Zurawski, Jr., and P.C. Hung. *In vitro* response to HBsAg of peripheral blood lymphocytes from recipients of hepatitis B vaccine. *Hepatology 4*:824–829, 1984.

Chen, D.-S., B.H. Hoyer, J. Nelson, R.H. Purcell, and J.L. Gerin. Detection and properties of hepatitis B virus DNA in liver tissues from patients with hepatocellular carcinoma. *Hepatology 2*:42S–46S, 1982.

Chen, D.-S., M.Y. Lai, and J.L. Sung. Delta agent infection in patients with chronic liver diseases and hepatocellular carcinoma—an infrequent finding in Taiwan. *Hepatology 4*:502–503, 1984.

Chien, M.C., M.J. Tong, K.J. Lo, J.K. Lee, D.R. Milich, G.N. Vyas, and B.L. Murphy. A study of hepatitis B viral markers in patients with primary hepatocellular carcinoma in Taiwan. *J. Nat. Cancer Instit. 66*:475–479, 1981.

Chin, T.W., F.B. Hollinger, R.R. Rich, C. Troisi, G.R. Dreesman, and J.L. Melnick. Cytotoxicity by NK-like cells from hepatitis B immune patients to a human hepatoma cell line secreting HBsAg. *J. Immunol. 130*:173–180, 1983.

Chisari, F.V., K.L. Castle, C. Xavier, and D.S. Anderson. Functional properties of lymphocyte subpopulations in hepatitis B virus infection. I. Suppressor cell control of T lymphocyte responsiveness. *J. Immunol. 126*:38–44, 1981.

Chisari, F.V., M.S. Bieber, C.A. Josepho, C. Xavier, and D.S. Anderson. Functional properties of lymphocyte subpopulations in hepatitis B virus infection. II. Cytotoxic effector cell killing of targets that naturally express hepatitis B surface antigen and liver-specific lipoprotein. *J. Immunol. 126*:45–49, 1981.

Christman, J.K., M. Gerber, P.M. Price, C. Flordellis, J. Edelman, and G. Acs. Amplification of expression of hepatitis B surface antigen in 3T3 cells contransfected with a dominant-acting gene and cloned viral DNA. *Proc. Natl. Acad. Sci. USA 79*:1815–1819, 1982.

Corey, L. and K.K. Holmes. Sexual transmissions of hepatitis A in homosexual men: incidence and mechanism. *N. Engl. J. Med. 302*:435–438, 1980.

Cote, P.J. Jr., R. Engle, C. Langer, A. Ponzetto, and J.L. Gerin. Antigenic analysis of woodchuck hepatitis virus surface antigen with site-specific radioimmunoassays. *J. Virol. 49*:701–708, 1984.

Cote, P.J., Jr., G.M. Dapolito, J.W.-K. Shih, and J.L. Gerin. Surface antigenic determinants of mammalian "hepadnaviruses" defined by group- and class-specific monoclonal antibodies. *J. Virol. 42*:135–142, 1982.

Cote, P.J., Jr. and J.L. Gerin. Non-overlapping antigenic sites of woodchuck hepatitis virus surface antigen and their cross-reactivity with surface antigens of the ground squirrel virus and hepatitis B virus surface antigens. *J. Virol. 47*:15–23, 1983.

Crivelli, O., M. Rizzetto, C. Lavarini, A. Smedile, and J.L. Gerin. Enzyme-linked immunosorbent assay for detection of antibody to the HBsAg-associated delta antigen. *J. Clin. Microbiol. 14*:173–177, 1981.

Cummings, I.W., J.K. Browne, W.A. Salser, G.V. Tyler, R.L. Snyder, J.M. Smolec, and J. Summers. Isolation, characterization, and comparison of recombinant DNAs derived from the human hepatitis B and woodchuck hepatitis virus genomes. *Proc. Natl. Acad. Sci. USA 77*:1842–1846, 1980.

Cupps, T.R., P.K. Goldsmith, D.J. Volkman, J.L. Gerin, R.H. Purcell, and A.S. Fauci. Activation of human peripheral blood B cells following immunization with hepatitis B surface antigen vaccine. *Cellular Immunology 86*:145–154, 1984.

Daemer, R.J., S.M. Feinstone, I.D. Gust, and R.H. Purcell. Propagation of human hepatitis A virus in African green monkey kidney cell culture: Primary isolation and serial passage. *Infect. Immun.* *32*:388–393, 1981.

Dawson, G.J., R.H. Decker, D.K. Norton, W.H. Bryce, R.O. Whittington, I.I. Tribby, and I.K. Mushahwar. Monoclonal antibodies to hepatitis A virus. *J. Med. Virol.* *14*:1–8, 1984.

de la Monte, S.M., G.M. Hutchins, and G.W. Moore. Risk factors development of lethal sequelae after hepatitis B virus infection in humans. *Am. J. Med.* *77*:482–488, 1984.

Denniston, K.J., T. Yoneyama, N. Sarver, B. Hoyer, and J.L. Gerin. Expression of hepatitis B virus genes cloned in bovine papillomavirus vectors. *Gene (in press)* 1984.

Dienstag, J.L., B.G. Werner, B.F. Polk, D.R. Snydman, D.E. Craven, R. Platt, C.S. Crumpacker, R. Quellet-Hellstrom, and G.F. Grady. Hepatitis B vaccine in health care personnel: safety, immunogenicity, and indicators of efficacy. *Ann. Intern. Med.* *101*:34–40, 1984.

Dienstag, J.L., C.E. Stevens, A.K. Bhan, and W. Szmuness. Hepatitis B vaccine administered to chronic carriers of hepatitis B surface antigen. *Ann. Intern. Med.* *96*:575–579, 1982.

Dreesman, G.R., F.B. Hollinger, Y. Sanchez, P. Oefinger, and J.L. Melnick. Immunization of chimpanzees with hepatitis B virus-derived polypeptides. *Infect. Immun.* *32*:62–67, 1981.

Dreesman, G.R., Y. Sanchez, I. Ionescu-Matiu, J.T. Sparrow, H.R. Six, D.L. Peterson, F.B. Hollinger, and J.L. Melnick. Antibodies to hepatitis B surface antigen after a single inoculation of uncoupled synthetic peptide. *Nature* *295*:158–160, 1982.

Dusheiko, G.M., J.H. Hoofnagle, W.G. Cooksley, S.P. James, and E.A. Jones. Synthesis of antibodies to hepatitis B virus by cultured lymphocytes from chronic hepatitis B surface antigen carriers. *J. Clin. Invest.* *71*:1104–1113, 1983.

Edman, J.C., P. Gray, P. Valenzuela, L.B. Rall, and W.J. Rutter. Integration of hepatitis B virus sequences and their expression in a human hepatoma cell. *Nature* *286*:535–538, 1980.

Edman, J.C., R. Hallewell, P. Valenzuela, H.M. Goodman, and W.J. Rutter. The synthesis of hepatitis B surface and core antigens in *E. coli*. *Nature* *291*:503–506, 1981.

Elfassi, E., J.L. Romet-Lemonne, M. Essex, M. Frances-McLanes, and W.A. Haseltine. Evidence of extrachromosomal forms of hepatitis B viral DNA in a bone marrow culture obtained from a patient recently infected with hepatitis B virus. *Proc. Natl. Acad. Sci. USA* *81*:3526–3528, 1984.

Engebretsen, B., A. Knight, and R. Shah. Hepatitis B in Southeast Asian refugees in Iowa. *Iowa Med.* *74*:105–108, 1984.

Feinman, S.V., L.R. Overby, B. Berris, K. Chau, C.A. Schable, and J.E. Maynard. The significance of IgM antibodies to hepatitis B core antigen in hepatitis B carriers and hepatitis B-associated chronic liver disease. *Hepatology* *2*:795–799, 1982.

Feinstone, S.M., H.J. Alter, H.P. Dienes, Y. Shimizu, H. Popper, D. Blackmore, D.L. Sly, W.T. London, and R.H. Purcell. Non-A, non-B hepatitis in chimpanzees and marmosets. *J. Infect. Dis.* *144*:588–598, 1981.

Feinstone, S.M., K.B. Mihalik, T. Kamimura, H.J. Alter, W.T. London, and R.H. Purcell. Inactivation of hepatitis B virus and non-A, non-B hepatitis by chloroform. *Infect. Immun.* *41*:816–821, 1983.

Feitelson, M.A., P.L. Marion, and W.S. Robinson. Antigenetic and structural relationships of the surface antigens of hepatitis B virus, ground squirrel hepatitis virus and woodchuck hepatitis virus. *J. Virol.* *39*:447–454, 1981.

Feitelson, M.A., P.L. Marion, and W.S. Robinson. The core particles of hepatitis B virus and ground squirrel hepatitis virus. I. Relationship between hepatitis B core antigen- and ground squirrel hepatitis core antigen-associated polypeptides by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and tryptic peptide mapping. *J. Virol.* *43*:687–696, 1982.

Feitelson, M.A., P.L. Marion, and W.S. Robinson. The core particles of hepatitis B virus and ground squirrel hepatitis virus. II. Characterization of the protein kinase reaction associated with ground squirrel hepatitis virus and hepatitis B virus. *J. Virol.* *43*:741–748, 1982.

Feitelson, M.A., P.L. Marion, and W.S. Robinson. The nature of polypeptides larger in size than the major surface antigen components of hepatitis B and like viruses in ground squirrels, woodchucks and ducks. *Virology* *130*:76–90, 1983.

Figus, A., H.E. Blum, G.N. Vyas, S. De Virgilis, A. Cao, M. Lippi, E. Lai, and A. Balestrieri. Hepatitis B viral nucleotide sequences in non-A, non-B or hepatitis B virus-related chronic liver disease. *Hepatology* *4*:364–368, 1984.

Francis, D.P., S.C. Hadler, S.E. Thompson, J.E. Maynard, D.D. Ostrow, N. Altman, E.N. Braff, P. O'Malley, D. Hawkins, F.N. Judson, K. Penley, T. Nylund, G. Christie, F. Meyers, J.N. Moore, Jr., A. Gardner, I.L. Doto, J.H. Miller, G.H. Reynolds, B.L. Murphy, C.A. Schable, B.T. Clark, J.W. Curran, and A.G. Redeker. The prevention of hepatitis B with vaccine. Report of the Centers for Disease Control multi-center efficacy trial among homosexual men. *Ann. Inter. Med.* *97*:362–366, 1982.

Ganem, D., B. Weiser, A. Barchuk, R.J. Brown, and H.E. Varmus. Biological characterization of acute infection with ground squirrel hepatitis virus. *J. Virol* *44*:366–373, 1982.

Ganem, D., L. Greenbaum, and H.E. Varmus. Virion DNA of ground squirrel hepatitis virus: structural analysis and molecular cloning. *J. Virol.* *44*:374–383, 1982.

Gerety, R.J., L.A. Smallwood, J.S. Finlayson, and E. Tabor. Standardization of the antibody to hepatitis A virus (anti-HAV) content of immunoglobulin. *Dev. Biol. Stand.* *54*:411–416, 1983.

Gerin, J.L., J.W.-K. Shih, R.H. Purcell, G. Dapolito, R. Engle, N. Green, H. Alexander, J.G. Sutcliffe, T.M. Shinnick, and R.A. Lerner. Chemically synthesized peptides of hepatitis B surface antigen duplicate the d/y specificities and induce sybtype-specific antibodies in chimpanzees. *Proc. Natl. Acad. Sci. USA 80*:2365–2396, 1983.

Gerlich, W.H. and W.S. Robinson. Hepatitis B virus contains protein attached to the 5' terminus of its complete DNA strand. *Cell 21*:801–809, 1980.

Gerlich, W.H., M.A. Feitelson, P.L. Marion, and W.S. Robinson. Structural relationships between the surface antigens of ground squirrel hepatitis virus and human hepatitis B virus. *J. Virol. 36*:787–795, 1980.

Gitnick, G., S. Weiss, L.R. Overby, C.M. Ling, R. Chairez, and K. Parsa. Non-A, non-B hepatitis: a prospective study of a hemodialysis outbreak with evaluation of a serologic marker in patients and staff. *Hepatology 3*:625–630, 1983.

Govindarajan, S., S.D. Lee, M.J. Tong, Y.T. Tsai, and K.J. Lo. Prevalence of delta agent among Chinese in Taiwan and Los Angeles. *J. Med. Virol. 14*:33–7, 1984.

Greenberg, H.B., W.S. Robinson, C.M. Knauer, and P.B. Gregory. Hepatitis B viral markers in severe viral hepatitis: Influence of steroid therapy. *Hepatology 1*:54–57, 1981.

Hadler, S.C., J.J. Erben, D.P. Francis, H.M. Webster, and J.E. Maynard. Risk factors for hepatitis A in day-care centers. *J. Infect. Dis. 145*:255–261, 1982.

Halpern, M.S., J.M. England, D.T. Deery, D.J. Petcu, W.S. Mason, and K.L. Molnar-Kimber. Viral nucleic acid synthesis and antigen accumulation in pancreas and kidney of Pekin ducks infected with duck hepatitis B virus. *Proc. Natl. Acad. Sci. USA 80*:4865–4869, 1983.

Halpern, M.S., J. Egan, W.S. Mason, and J.M. England. Viral antigen in endocrine cells of the pancreatic islets and adrenal cortex of Pekin ducks infected with duck hepatitis B virus. *Virus Res. 1*:213–223, 1984.

Halpern, M.S., J.M. England, L. Flores, J. Egan, J. Newbold, and W.S. Mason. Individual cells in liver, kidney, and pancreas of DHBV infected ducks express antigens cross-reactive with those on viral surface antigen particles and immature virus cores. *Virology 137*:408–413, 1984.

Hann, H.W., C.Y. Kim, W.T. London, P. Whitford, and B.S. Blumberg. Hepatitis B virus and primary hepatocellular carcinoma: family studies in Korea. *Int. J. Cancer 30*: 47–51, 1982.

Hanson, R.G., J.H. Hoofnagle, G.Y. Minuk, R.H. Purcell, J.L. Gerin. Cell-mediated immunity to hepatitis B surface antigen in man. *Clin. Exptl. Immunol. 57*:257–264, 1984.

Hirschman, S.Z., P. Price, E. Garfinkel, J. Christman, and G. Acs. Expression of cloned hepatitis B virus DNA in human cell cultures. *Proc. Natl. Acad. Sci. USA 77*:5507–5511, 1980.

Hollinger, F.B., G. Dolana, W. Thomas, and F. Gyorkey. Reduction of risk of hepatitis transmission by heat-treatment of a human factor VIII concentrate. *J. Infect. Dis. 150*:250–262, 1984.

Hollinger, F.B., J.W. Mosley, W. Szmuness, R.D. Aach, R.L. Peters, and C. Stevens. Transfusion-transmitted viruses study: experimental evidence for two non-A, non-B hepatitis agents. *J. Infect. Dis. 142*:400–407, 1980.

Hoofnagle, J.H., G.Y. Minuk, G.M. Dusheiko, D.F. Schafer, R. Johnson, S. Straus, E.A. Jones, J.L. Gerin, and K. Ishak. Adenine arabinoside-5'-monophosphate for the treatment of chronic type B hepatitis. *Hepatology 2*:784–788, 1982.

Hoofnagle, J.H., G.M. Dusheiko, L.B. Seeff, E.A. Jones, J.G. Waggoner, and Z.B. Bales. Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. *Ann. Intern. Med. 94*, 1981.

Hoofnagle, J.H. and L.B. Seeff. Natural history of chronic type B hepatitis. *Prog. Liver Dis. 7*:469–479, 1982.

Hoofnagle, J.H., G.M. Dusheiko, D.F. Schafer, E.A. Jones, K.C. Micetich, R.C. Young, and J. Costa. Reactivation of chronic hepatitis B virus infection by cancer chemotherapy. *Ann. Intern. Med. 96*:447–449, 1982.

Hoyer, B., F. Bonino, A. Ponzetto, K. Denniston, J. Nelson, R. Purcell, and J.L. Gerin. Properties of delta-associated ribonucleic acid. *Prog. Clin. Biol. Res. 143*:91–97, 1983.

Ionescu-Matiu, I., Y. Sanchez, F.B. Hollinger, J.L. Melnick, and G.R. Dreesman. Presence of a receptor for albumin on individual HBsAg polypeptides and on HBsAg produced by a hepatoma cell line. *J. Med. Virol. 6*:175–178, 1980.

Ionescu-Matiu, I., R.C. Kennedy, J.T. Sparrow, A.R. Culwell, Y. Sanchez, J.L. Melnick, and G.R. Dreesman. Epitopes associated with a synthetic hepatitis B surface antigen peptide. *J. Immunol. 130*:1947–1952, 1983.

Kam, W., L.B. Rall, E.A. Smuckler, R. Schmid, and W.J. Rutter. Hepatitis B viral DNA in the liver and serum of asymptomatic carriers. *Proc. Natl. Acad. Sci. USA 79*:7522–7526, 1982.

Kamimura, T., A. Ponzetto, F. Bonino, S.M. Feinstone, J.L. Gerin, and R.H. Purcell. Cytomplasmic tubular structures in liver of HBsAg carrier chimpanzees infected with delta agent and comparison with cytomplasmic structures in non-A, non-B hepatitis. *Hepatology 5*:631–637, 1983.

Kanel, G.C., S. Govindarajan, and R.L. Peters. Chronic delta infection and liver biopsy changes in chronic active hepatitis B. *Ann. Intern. Med. 101*:51–54, 1984.

Kao, H.W., M. Ashcavai, and A.G. Redeker. The persistence of hepatitis A IgM antibody after acute clinical hepatitis A. *Hepatology 4*:933–936, 1984.

Katz, D., J.L. Melnick, and F.B. Hollinger. Characterization of HBeAg by physiochemical and immunochemical methods. *J. Med. Virol.* *5*:67–100, 1980

Kennedy, R.C., Y. Sanchez, I. Ionescu-Matiu, J.L. Melnick, and G.R. Dreesman. A common human anti-hepatitis B surface antigen idiotype is associated with the group *a* conformation-dependent antigenic determinant. *Virology* *122*:219–221, 1982

Kennedy, R.C. and G.R. Dreesman. Common idiotypic determinant associated with human antibodies to hepatitis B surface antigen. *J. Immunol.* *130*:385–389, 1983

Kennedy, R.C., I. Ionescu-Matiu, K. Adler-Storthz, R.D. Henkel, Y. Sanchez, and G.R. Dreesman. Characterization of anti-hepatitis B surface antigen monoclonal antibodies. *Intervirology* *19*:176–180, 1983

Kennedy, R.C., I. Ionescu-Matiu, Y. Sanchez, and G.R. Dreesman. Detection of interspecies idiotypic cross-reactions associated with antibodies to hepatitis B surface antigen. *Eur. J. Immunol.* *13*:232–235, 1983

Kennedy, R.C., G.R. Dreesman, J.T. Sparrow, A.R. Culwell, Y. Sanchez, I. Ionescu-Matiu, F.B. Hollinger, and J.L. Melnick. Inhibition of a common human anti-hepatitis B surface antigen idiotype by a cyclic synthetic peptide. *J. Virol.* *46*:653–655, 1983

Kennedy, R.C., K. Adler-Storthz, R.D. Henkel, Y. Sanchez, J.L. Melnick, and G.R. Dreesman. Immune response to hepatitis B surface antigen: enhancement by prior injection of anti-idiotype antibodies. *Science* *221*:853–855, 1983

Kennedy, R.C, J.L. Melnick, and G.R. Dreesman. Viral hepatitis B antibody (anti-HBs) induced by injecting anti-idiotype antibodies. *Science* *223*:930–931, 1984

Kennedy, R.C. and G.R. Dreesman. Enhancement of the immune response to hepatitis B surface antigen: *in vivo* administration of anti-idiotype induces anti-HBs which expresses a similar idiotype. *J. Exp. Med.* *159*:655–665, 1984

Kennedy, R.C., J.T. Sparrow, Y. Sanchez, J.L. Melnick, and G.R. Dreesman. Enhancement of viral hepatitis B antibody (anti-HBs) response to a synthetic cyclic peptide by priming with anti-idiotype antibodies. *Virology* *136*:247–252, 1984

Khan, N.C., F.B. Hollinger, and J.L. Melnick. Localization of hepatitis A virus antigen to specific subcellular fractions of hepatitis A infected chimpanzee liver cells. *Intervirology* *21*:187–194, 1984

Krawczynski, K.K., D.W. Bradley, B.L. Murphy, J.W. Ebert, T.E. Anderson, I.L. Doto, A. Nowoslawski, W. Duermeyer, and J.E. Maynard. Pathogenetic aspects of hepatitis A virus infection in enterally inoculated marmosets. *Am. J. Clin. Pathol.* *76*:698–706, 1981

Krugman, S., H.P. Holley, Jr., M. Davidson, M.S. Simberkoff, and N. Matsaniotis. Immunogenic effect of inactivated hepatitis B vaccine: comparison of 20 microgram and 40 microgram doses. *J. Med. Virol.* *8*:119–121, 1981

Langendorfer, A., W. Davenport, W.T. London, B.S. Blumberg, and S. Mazzur. Sex-related differences in transmission of hepatitis B infection in a Melanesian population. *Am. J. Phys. Anthropol.* *64*:243–254, 1984

Laub, O., L.B. Rall, M. Truett, Y. Shaul, D.N. Standring, P. Valenzuela, and W.J. Rutter. Synthesis of hepatitis B surface antigen in mammalian cells: Expression of the entire gene and the coding region. *J. Virol.* *48*:271–280, 1983

Lemon, S.M., R.M. Scott, and W.H. Bancroft. Subcutaneous administration of inactivated hepatitis B vaccine by automatic jet injection. *J. Med. Virol.* *12*:129–136, 1983

Lemon, S.M. and L.N. Binn. Antigenic relatedness of two strains of hepatitis A virus determined by cross-neutralization. *Infect. Immun.* *42*:418–420, 1983

Lemon, S.M., L.N. Binn, and R.H. Marchwicki. Radioimmunofocus assay for quantitation of hepatitis A virus in cell cultures. *J. Clin. Microbiol.* *17*:834–839, 1983

Lemon, S.M., N.L. Gates, T.E. Simms, and W.H. Bancroft. IgM antibody to hepatitis B core antigen as a diagnostic parameter of acute infection with hepatitis B virus. *J. Infect. Dis.* *143*:803–809, 1981

Lerner, R.A., N. Green, H. Alexander, F.T. Liu, J.G. Sutcliffe, and T.M. Shinnick. Chemically synthesized peptides predicted from the nucleotide sequence of the hepatitis B virus genome elicit antibodies reactive with the native envelope protein of Dane particles. *Proc. Natl. Acad. Sci. USA* *78*:3403–3407, June 1981

Lindsay, K.L., A.G. Redeker, and M. Ashcavai. Delayed HBsAg clearance in chronic hepatitis B viral infection. *Hepatology* *1*:586–589, 1981

Marion, P.L., F.H. Salazar, J.J. Alexander, and W.S. Robinson. The state of hepatitis B viral DNA in a human hepatoma cell line. *J. Virol.* *33*: 795–806, 1980

Marion, P.L., L.S. Oshiro, D.C. Regnery, G.H. Scullard, and W.S. Robinson. A virus in Beechey ground squirrels that is related to hepatitis B virus of humans. *Proc. Natl. Acad. Sci. USA* *77*:2941–2945, 1980

Marion, P.L., S.S. Knight, F.H. Popper, and W.S. Robinson. Ground squirrel hepatitis virus infection. *Hepatology* *3*:519–527, 1983

Marion, P.L., S.S. Knight, M.A. Feitelson, L.S. Oshiro, and W.S. Robinson. The major polypeptide of duck hepatitis B surface antigen particles. *J. Virol.* *48*:534–541, 1983

Marion, P.L., S.S. Knight, B-K Ho, Y-Y Guo, W.S. Robinson, and H. Popper. Liver disease associated with duck hepatitis B virus infection of domestic ducks. *Proc. Natl. Acad. Sci. USA* *81*:898–902, 1984

Mason, W.W., G. Seal, and J. Summers. A virus of Pekin ducks with structural and biological relatedness to human hepatitis B virus. *J. Virol. 36*:829–836, 1980.

Mason, W.S., C. Aldrich, J. Summers, and J.M. Taylor. Asymmetric replication of duck hepatitis B virus DNA in liver cells (free minus strand DNA). *Proc. Natl. Acad. Sci. USA 79*:3997–4001, 1982.

Mason, W.W., M.S. Halpern, J.M. England, G. Seal, J. Egan, L. Coates, C. Aldrich, and J. Summers. Experimental transmission of duck hepatitis B virus. *Virology 131*:375–384, 1983.

Mathiesen, L.R., P. Skinhoj, J.O. Nielsen, R.H. Purcell, D. Wong, and L. Ranek. Hepatitis type A, B, and non-A, non-B in fulminant hepatitis. *GUT 21*:72–77, 1980.

McAleer, W.J., H.Z. Markus, D.E. Wampler, E.B. Bunak, W.J. Miller, R.E. Weibel, A.A. McLean, and M.R. Hilleman. Vaccine against human hepatitis B virus prepared from antigen derived from human hepatoma cells in culture. *Proc. Soc. Exp. Biol. Med. 175*:314–319, 1984.

McAuliffe, V.J., R.H. Purcell, J.L. Gerin, and F.J. Tyeryar (Eds., W. Szmuness, H. Alter, and J. Maynard). Current status of the NIAID hepatitis B vaccines. *Viral Hepatitis 1981 International Symposium*:425–435, 1982.

Mclean, A.A., M.R. Hilleman, W.J. McAleer, and E.B. Buynak. Summary of worldwide clinical experience with H-B-Vax (B, MSD). *J. Infect. 7 Suppl 1*:95–104, 1983.

Milich, D.R. and F.V. Chisari. Genetic regulation of the immune response to hepatitis B surface antigen (HBsAg) I. H-2 restriction of the murine humoral immune response to the *a* and *d* determinants of HBsAg. *J. Immunol. 129*:320–325, 1982.

Milich, D.R., G.G. Leroux-Roels, and F.V. Chisari. Genetic regulation of the immune response to hepatitis B surface antigen (HBsAg). II. Qualitative characteristics of the humoral immune response to the *a*, *d*, and *y* determinants of HBsAg. *J. Immunol. 130*:1395, 1983.

Milich, D.R., H. Alexander, and F.V. Chisari. Genetic regulation of the immune response to hepatitis B surface antigen (HBsAg). III. Circumvention of nonresponsiveness in mice bearing nonresponder haplotypes. *J. Immunol. 130*:1401, 1983.

Milich, D.R., T.D. Gottfried, and G.N. Vyas. Characterization of the interaction between polymerized human albumin and hepatitis B surface antigen. *Gastroenterol. 81*:218–225, 1981.

Milich, D.R., P.K. Bhatnagar, E. Papas, and G.N. Vyas. Interactions between polymerized human albumin, hepatitis B surface antigen and complement: I. Binding of polyalbumin to $C1_q$. *J. Med. Virol. 7(3)*:181–192, 1981.

Milich, D.R., P.K. Bhatnagar, and G.N. Vyas. Interactions between polymerized human albumin, hepatitis B surface antigen and complement: II. Involvement of $C1_q$ in or near the hepatitis B surface antigen receptor for human polymerized albumin. *J Med. Virol. 7(3)*:1983–204, 1981.

Milich, D.R., G.G. Leroux-Roels, and F Chisari. Genetic regulation of the immune response to hepatitis B surface antigen (HBsAg): IV. Distinct H-2-linked Ir genes control antibody responses to different HBsAg determinants on the same molecule and map to the I-A and I-C subregions. *J. Exp. Med. 159*:41, 1984.

Miller, R.H. and W.S. Robinson. Integrated hepatitis B virus DNA sequences specifying the major viral core polypeptide are methylated in PLC/PRF/5 cells. *Proc. Natl. Acad. Sci. USA 80*:2534–2538, 1983.

Miller, R.H. and W.S. Robinson. Hepatitis B viral DNA forms in nuclear and cytoplasmic fractions of infected human liver. *Virology 137*:390–399, 1984.

Miller, R.H., P.L. Marion, and W.S. Robinson. Hepatitis B viral DNA-RNA hybrid molecules in particles from infected liver are converted to viral DNA molecules during an endogenous DNA polymerase reaction. *Virology 139*:64–72, 1984.

Miller, R.H., C.T. Tran, and W.S. Robinson. Hepatitis B virus particles of plasma and liver contain viral DNA-RNA hybrid molecules. *Virology 139*:53–63, 1984.

Millman, I., T. Halbherr, and H. Simmons. Immunological cross-reactivities of woodchuck and hepatitis B viral antigens. *Infect. Immun. 35*:752–757, 1982.

Minuk, G.Y., J.H. Hoofnagle, V.J. McAuliffe, and R.H. Purcell. Immunoglobulin M (IgM) antibody responses to hepatitis B surface antigen in recipients of the hepatitis B virus vaccine. *J. Infect. Dis. 148*:330, 1983.

Mitamura, K., G.H. Hoyer, A. Ponzetto, J. Nelson, R.H. Purcell, and J.L. Gerin. Woodchuck hepatitis virus DNA in woodchuck liver tissues. *Hepatology 2*:47S–50S, 1982.

Molnar-Kimber, K.L., J. Summers, J.M. Taylor, and W.S. Mason. Protein is covalently bound to minus strand DNA intermediates of duck hepatitis B virus. *J. Virol. 45*:165–172, 1983.

Molnar-Kimber, K., J. Summers, and W.S. Mason. Mapping of the cohesive overlap of duck hepatitis B virus DNA and of the site of initiation of reverse-transcription. *J. Virol. 51*:181–191, 1984.

Moriarty, A.M., B.H. Hoyer, J.W.-K. Shih, J.L. Gerin, and D.H. Hamer. Expression of the hepatitis B virus surface antigen gene in cell culture using a simian virus 40 vector. *Proc. Natl. Acad. Sci. 78*:2606–2610, 1981.

Mosley, J.W., K.A. Visona, and V.M. Villarejos. Immunoglobulin M level in the diagnosis of type A hepatitis. *Amer. J. Clin. Pathol. 75*:86–87, 1981.

Moss, B., G.L. Smith, J.L. Gerin, and R.H. Purcell. Live recombinant vaccinia virus protects chimpanzees against hepatitis B. *Nature 311*:67–69, 1984.

Mushahwar, I.K., J.L. Gerin, J.L. Dienstag, R.H. Decker, A. Smedile, and M. Rizzetto. Interpretation of Hepatitis B Virus and Hepatitis Delta Virus Serologic Profiles. *Pathologist 38*:648–650, 1984.

Nath, N., C.T. Fang, H.A. Fields, I.L. Doto, and J.E. Maynard. Distribution of hepatitis B e antigen (HBeAg) and anti-HBe in carriers with different levels of HBsAg. *J. Med. Virol. 6*:179–184, 1980.

Neurath, A.R., S.B. Kent, and N. Strick. Location and chemical synthesis of a pre-S gene coded immunodominant epitope of hepatitis B virus. *Science 224*:392–395, 1984.

Oefinger, P.E., D.L. Bronson, and G.R. Dreesman. Induction of hepatitis B surface antigen in human hepatoma-derived cell lines. *J. Gen. Virol. 6*:175–178, 1980.

Ogston, W., G. Jonak, C. Rogler S. Astrin, and J. Summers. Cloning and structural analysis of integrated woodchuck hepatitis virus sequences from hepatocellular carcinomas of woodchucks. *Cell 29*:385–394, 1982.

Palla, M., R. Rizzi, M. Toti, P. Almi, M. Rizzetto, F. Bonino, and R.H. Purcell. Complexes of HBsAg and IgM in the serum of patients with hepatitis B virus infection: further characterization. *Infect. Immun. 41*:950–958, 1983.

Paoletti, E., B.R. Lipinskas, C. Samsonoff, S. Mercer, and D. Panicali. Construction of live vaccines using genetically engineered poxviruses: biological activity of vaccinia virus recombinants expressing the hepatitis B virus surface antigen and the herpes simplex virus glycoprotein D. *Proc. Natl. Acad Sci. USA 81*:193–197, 1984.

Papaevangelou, G., A. Roumeliotou-Karayannis, C. Vissoulis, P. Stathopoulou, N. Kolaitis, and S. Krugman. Reduction of the dose of hepatitis B vaccine. *J. Infect. 7 Suppl 1*:69–70, 1983.

Papaevangelou, G.J., C.G. Vissoulis, A.J. Roumeliotou-Karayannis, N. Kolaitis, and S. Krugman. Comparison of safety and immunogenicity of ADW and AYW hepatitis B vaccines. *J. Med. Virol.* 9:231-236, 1982.

Peterson, D.L. Isolation and characterization of the major protein and glycoprotein of hepatitis B surface antigen. *J. Biol. Chem. 256*:6975–6983, 1981.

Peterson, D.L., N. Nath, and F. Gavilanes. Structure of hepatitis B surface antigen: Correlation of subtype with the amino acid sequence and location of the carbohydrate moiety *J. Biol. Chem. 257*:10414–10420, 1982.

Peterson, D.L., D.A. Paul, J. Lam, and D.T. Achord. Antigen structure of hepatitis B surface antigen: Identification of the "d" subtype determinant by chemical modification and use of monoclonal antibodies. *J. Immuno. 132*:920–927, 1984

Ponzetto, A., P.J. Cote, Jr., H. Popper, B.H. Hoyer, W.T London, E.C. Ford, F. Bonino, R.H. Purcell, and J.L. Gerin. Transmission of the hepatitis B virus associated delta agent to the eastern woodchuck. *Proc. Natl. Acad. Sci. USA 81*:2208–2212, 1984.

Ponzetto, A., P.J. Cote, Jr., E.C. Ford, R.H. Purcell, and J.L. Gerin. Core antigen and antibody in woodchucks after infection with woodchuck hepatitis virus. *J. Virol. 52*:70–76, 1984.

Popper, H., J.W.-K. Shih, J.L. Gerin, D.C. Wong, B.H. Hoyer, W.T. London, D.L. Sly, and R.H. Purcell. Woodchuck hepatitis and hepatocellular carcinoma: correlation of histologic with virologic observations. *Hepatology 1*:91–98, 1981.

Popper, H., S.N. Thung, M.A. Gerger, S.C. Hadler, M. DeMonzon, A. Ponzetto, E. Anzola, D. Rivera, A. Mondolfi, A. Bracho, D.P. Francis, J.L. Gerin, J.E. Maynard, and R.H. Purcell. Histologic studies of severe delta agent infection in Venezuelan Indians. *Hepatology 3*:906–912, 1983.

Popper, H., J.L. Dienstag, S.M. Feinstone, H.J. Alter, and R.H. Purcell. The pathology of viral hepatitis in chimpanzees. *Virchows Archiv. A. Pathology, Anatomy and Histology 387*:91–106, 1980.

Price, P., S. Ostrove, C. Flordellis, M.A. Sells, S. Thung, M. Gerber, J. Christman, and G. Acs. Characterization of RNA transcripts and virally coded proteins synthesized in mouse fibroblasts transfected with hepatitis B DNA: HBeAg synthesis in HBcAg-negative cells with active core-antigen genes. *Biosci. Rep. 3*:1017–1026, 1983.

Price, P., S.Z. Hirschman, and E. Garfinkel. DNA cloned from the ayw subtype of hepatitis B virus. *J. Med. Virol. 6*:139–145, 1980.

Prince, A.M., T. Huima, B.A.A. Williams, L. Bardina, and B. Brotman. Isolation of a virus from chimpanzee liver cell cultures inoculated with sera containing the agent of non-A, non-B hepatitis. *Lancet 2*:1071–1074, 1984.

Prince, A.M., W. Stephen, and B. Brotman. Inactivation of non-A, non-B virus infectivity by a beta propiolactone/ultraviolet irradiation treatment and aerosil absorption procedure for preparation of a stabilized human serum. *Vox Sang. 46*:80–85, 1984.

Provost, P.J., P.A. Conti, P.A. Giesa, F.S. Banker, E.B. Buynak, W.J. McAleer, and M.R. Hilleman. Studies in chimpanzees of live, attenuated hepatitis A vaccine candidates. *Proc. Soc. Exp. Biol. Med. 172*:357–363, 1983.

Provost, P.J., F.S. Banker, P.A. Giesa, W.J. McAleer, E.B. Buynak, and M.R. Hilleman. Progress toward a live, attenuated human hepatitis A vaccine. *Proc. Soc. Exp. Biol. Med. 170*:8-14, 1982.

Provost, P.J., P.A. Giesa, W.J. McAleer, and M.R. Hilleman. Isolation of hepatitis A virus *in vitro* in cell culture directly from human specimens. *Proc. Soc. Exp. Biol. Med. 167*:201–206, 1981

Prozesky, O.W., C.E. Stevens, W. Szmuness, H. Rolka, E.J. Harley, M.C. Kew, J.E. Scholtz, and A.D. Mitchell. Immune response to hepatitis B vaccine in newborns. *J. Infect. 7 Suppl 1*:53–55, 1983

Puerrillo, R., C. Campbell, W. Wellinghoff, and L. Gelb. The relationship of hepatitis B e antigen, DNA polymerase activity, and titer of hepatitis B surface antigen with ongoing liver injury in chronic hepatitis B virus infection. *Am. J. Gastroenterol.* *77*:445–449, 1982.

Rall, L.B., D.M. Standring, W. Laub, and W.J. Rutter. Transcription of hepatitis B virus by RNA polymerase II. *Mol. Cell. Bio.* *3*:1766–1773, 1983.

Rizzetto, M., M.G. Canese, J.L. Gerin, W.T. London, D.L. Sly, and R.H. Purcell. Transmission of the hepatitis B virus-associated delta antigen to chimpanzees. *J. Infect. Dis.* *141*:590–602, 1980.

Rizzetto, M., R.H. Purcell, and J.L. Gerin. Epidemiology of HBV-associated delta agent: geographical distribution of anti-delta and prevalence in polytransfused HBsAg carriers. *Lancet* *1*:1215–1218, 1980.

Rizzetto, M., J.W.-K. Shih, and J.L. Gerin. The hepatitis B virus-associated δ antigen: isolation from liver, development of solid-phase radioimmunoassays for antigen and antibody and partial characterization of antigen. *J. Immunol.* *125*:318–324, 1980.

Rizzetto, M., B. Hoyer, M.G. Canese, J.W.-K. Shih, R.H. Purcell, and J.L. Gerin. Delta agent: the association of delta antigen with hepatitis B surface antigen and ribonucleic acid in the serum of delta-infected chimpanzees. *Proc. Natl. Acad. Sci. USA* *77*:6124–6128, 1980.

Rizzetto, M., J.W.-K. Shih, G. Verme, and J.L. Gerin. A radioimmunoassay for HBcAg in the sera of HBsAg carriers: serum HBcAg, serum DNA polymerase activity and liver HBcAg immunofluorescence as markers of chronic liver disease. *Gastroenterology* *80*:1420–1427, 1981.

Rizzetto, M., M.G. Canese, R.H. Purcell, W.T. London, L.D. Sly, and J.L. Gerin. Experimental HBV and δ infections of chimpanzees: occurrence and significance of intrahepatic immune complexes of HBcAg and antigen. *Hepatology* *1*:567–574, 1981.

Rogler, C.E., and J. Summers. Novel forms of woodchuck hepatitis virus DNA isolated from chronically infected woodchuck liver nuclei. *J. Virol.* *44*:852–863, 1982.

Rogler, C.E., and J. Summers. Cloning and structural analysis of integrated woodchuck hepatitis virus sequences from chronically infected liver. *J. Virol.* *50*:832–837, 1984.

Roth, L., J.M. King, W.E. Hornbuckle, H.J. Harvey, and B.C. Tennant. Chronic hepatitis and hepatoma associated with persistent woodchuck hepatitis virus infection. *Vet. Path.* (in press), 1984.

Ruiz-Opazo, N., P.R. Chakraborty, and D.A. Shafritz. Evidence for supercoiled hepatitis B virus DNA in chimpanzee liver and serum Dane particles: possible implications in persistent HBV infection. *Cell* *29*:129–136, 1982.

Sacks, S.L., G.H. Scullard, R.B. Pollard, P.B. Gregory, W.S. Robinson, and T.C. Merigan. Antiviral treatment of chronic hepatitis B virus infection: Pharmacokinetics and side effects of interferon and adenine arabinoside alone and in combination. *Antimicrobiol Agents and Chemotherapy* *21*:93–100, 1982.

Sampliner, R.E., B.L. Loevinger, E. Tabor, and R.J. Gerety. Intrafamilial cluster of hepatitis B virus infection: study of a large family in the United States. *Am. J. Epidemiol.* *113*:50–54, 1981.

Sanchez, Y., I. Ionescu-Matiu, G.R. Dreesman, W. Kramp, H.R. Six, F.B. Hollinger, and J.L. Melnick. Humoral and cellular immunity to hepatitis B virus-derived antigens: comparative activity of Freund complete adjuvant, alum, and liposomes. *Infec. Immun.* *30*:728–733, 1980.

Sanchez, Y., I. Ionescu-Matiu, F.B. Hollinger, J.L. Melnick, and G.R. Dreesman. Antigenic relationship of a hepatitis B surface antigen-derived polypeptide and human serum albumin. *J. Gen. Virol.* *48*:273–283, 1980.

Sanchez, Y., I. Ionescu-Matiu, G.R. Dreesman, F.B. Hollinger, and J.L. Melnick. Evidence for the presence of repeating antigenic determinants in the major and minor polypeptides derived from hepatitis B surface antigen. *Virology* *114*:71–81, 1981.

Sanchez, Y., I. Ionescu-Matiu, J.T. Sparrow, J.L. Melnick, and G.R. Dreesman. Immunogenicity of conjugates and micelles of synthetic hepatitis B surface antigen peptides. *Intervirology* *18*:209–213, 1982.

Sanchez, Y., I. Ionescu-Matiu, J.L. Melnick, and G.R. Dreesman. Comparative studies of the immunogenic activity of hepatitis B surface antigen (HBsAg) and HBsAg polypeptides. *J. Med. Virol.* *11*:115–124, 1983.

Schaeffer, E., and J.J. Sninsky. Predicted secondary structure similarity in the absence of primary amino acid sequence homology: hepatitis B virus open reading frames. *Proc. Natl. Acad. Sci. USA* *81*:2902–2906, 1984.

Schaff, Z., E. Tabor, D.R. Jackson, and R.J. Gerety. Ultrastructural alterations in serial liver biopsy specimens from chimpanzees experimentally infected with a human non-A, non-B hepatitis agent. *Virchows Arch. [Cell Pathol.]* *45*:301–312, 1984.

Schreeder, M.T., S.E. Thompson, S.C. Hadler, K.R. Berquist, A. Zaidi, J.E. Maynard, D. Ostrow, F.N. Judson, E.H. Braff, T. Nylund, J.N. Moore, Jr., P. Gardner, I.L. Doto, and G. Reynolds. Hepatitis B in homosexual men: prevalence of infection and factors related to transmission. *J. Infect. Dis.* *146*:7–15, 1982.

Scott, R.M., R. Snitghan, W.H. Bancroft, H.J. Alter, and M. Tingpalapong. Experimental transmission of hepatitis B virus by semen and saliva. *J. Infect. Dis.* *142*:67–71, 1980.

Scullard, G.H., C.I. Smith, T.C. Merigan, W.S. Robinson, and P.B. Gregory. Effects of immunosuppressive therapy on viral markers in chronic active hepatitis B. *Gastroenterol. 81*:987–991, 1981.

Scullard, G.H., R.B. Pollard, J.L. Smith, S.L. Sacks, P.B. Gregory, W.S. Robinson, and T.C. Merigan. Antiviral treatment of chronic hepatitis B virus infection. I. Changes in viral markers with interferon combined with adenine arabinoside. *J. Infect. Dis. 143*:772–783, 1981.

Scullard, G.H., H.B. Greenberg, J.L. Smith, P.B. Gregory, T.C. Merigan, and W.S. Robinson. Antiviral treatment of chronic hepatitis B virus infection: Infectious virus cannot be detected in patient serum after permanent responses to treatment. *Hepatology 2*:39–49, 1982.

Seeger, C., D. Ganem, and H.E. Varmus. The cloned genome of ground squirrel hepatitis virus is infectious in the animal. *Proc. Natl. Acad. Sci. USA 81*:5849–5852, 1984.

Seeger, C., D. Ganem, and H.E. Varmus. Nuceolotide sequence of an infectious, molecularly cloned genome of the ground squirrel hepatitis B virus. *J. Virol. 51*:367–375, 1984.

Serrano, M.A., and S.Z. Hirschman. Properties of hepatitis B antigen synthesized by rat cells transfected with circular viral DNA. *J. Gen. Virol. 65*:1373–1383, 1984.

Seto, B., W.G. Coleman, S. Iwarson, and R.J. Gerety. Detection of reverse transcriptase activity in association with the non-A, non-B hepatitis agent(s). *Lancet 2*:941–943, 1984.

Shafritz, D.A., H.M. Lieberman, K.J. Isselbacher, and J.R. Wands. Monoclonal radioimmunoassays for hepatitis B surface antigen: demonstration of hepatitis B virus DNA or related sequences in serum and viral epitopes in immune complexes. *Proc. Natl. Acad. Sci. USA 79*:5675–5679, 1982.

Shafritz, D.A. Integration of HBV-DNA into liver and hepatocellular carcinoma cells during persistent HBV infection. *J. Cell. Biochem. 20*:303–316, 1982.

Shafritz, D.A., D. Shouval, H.I. Sherman, S.J. Hadziyannis, and M.C. Kew. Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepatocellular carcinoma. Studies in percuteneous liver biopsies and postmortem tissue specimens. *N. Engl. J. Med. 305*:1067–1073, 1981.

Shaul, Y., M. Ziemer, P.D. Garcia, R. Crawford, H. Hsu, P. Valenzuela, and W.J. Rutter. Cloning and analysis of integrated hepatitis virus sequences from a human hepatoma cell line. *J. Virol. 51*:776–787, 1984.

Shih, J.W.-K., P.L. Tan, and J.L. Gerin. Relationship of large hepatitis B surface antigen polypeptide to human serum albumin. *Infection and Immunity 28*:459–463, 1980.

Shih, J.W.-K., G. Hess, P.M. Kaplan, and J.L. Gerin. Characterization of hepatitis B virus (Dane particle). *J. Virol Meth. 1*:47–59, 1980.

Shih, J.W.-K., P.J. Cote, Jr., G.M. Dapolito, and J.L. Gerin. Production of monoclonal antibodies against HBsAg by somatic cell hybrids. *Virol. Meth. 1*:257–273, 1980.

Shih, M.F., M. Arsenakis, P. Tiollais, and B. Roizman. Expression of hepatitis B virus S gene by herpes simplex virus type 1 vectors carrying alpha- and beta-regulated gene chimeras. *Proc. Natl. Acad. Sci. USA 81*:5867–5870, 1984.

Shouval, D., R. Ross, L.M. Reid, and D.A. Shafritz. Enhanced tumorigenicity and hepatitis B virus genes in a human hepatoma cell line injected into immunosuppressed nude mice. *Trans. Assoc. Am. Physicians 94*:154–164, 1981.

Shouval, D., L.M. Reid, P.R. Chakraborty, N. Ruiz-Opazo, R. Morecki, M.A. Gerber, S.M. Thung, and D.A. Shafritz. Tumorigenicity in nude mice of a human hepatoma cell line containing hepatitis B virus DNA. *Cancer Res. 41*:1342–1350, 1981.

Shouval, D., P.R. Chakraborty, N. Ruiz-Opazo, S. Baum, I. Spigland, E. Muchmore, M.A. Gerber, S.N. Thung, H. Popper, and D.A. Shafritz. Chronic hepatitis in chimpanzee carriers of hepatitis B virus: morphologic, immunologic, and viral DNA studies. *Proc. Natl. Acad. Sci. USA 77*:6147–6151, 1980.

Siddiqui, A. Expression of Hepatitis B surface antigen in cultured cells by using recombinant plasmid vectors. *Mol. Cell. Biol. 3*:143, 1983.

Siddiqui, A. Hepatitis B virus DNA in Kaposi Sarcoma. *Proc. Natl. Acad. Sci. USA 80*:4861–4864, 1983.

Siddiqui, A., P.L. Marion, and W.S. Robinson. Ground squirrel hepatitis virus DNA: Molecular cloning and comparison with hepatitis B virus DNA. *J. Virol. 38*:393–397, 1981.

Simonsen, C.C., and A.D. Levinson. Analysis of processing and polyadenylation signals of the hepatitis B virus surface antigen gene by using simian virus 40-hepatitis B virus chimeric plasmids. *Bol. Cell. Biol. 3*:2250–2258, 1983.

Smith, C.I., L.W. Kitchen, G.H. Scullard, W.S. Robinson, P.B. Gregory, and T.C. Merigan. Vidarabine monophosphate and human leukocyte interferon in chronic hepatitis B infection. *J. Am. Med. Assoc. 247*:2261–2265, 1982.

Smith, C.I., J. Weissberg, P.B.B. Gregory, W.S. Robinson, L. Bernhardt, and T.C. Merigan. Acute Dane particle suppression with recombinant leukocyte A interferon in chronic hepatitis B virus infection. *J. Infect. Dis. 148*:907–913, 1983.

Smith, C.I., and T.C. Merigan. Therapeutic approaches to chronic hepatitis B. *Prog. Liver Dis. 7*:481–594, 1982.

Smith, G.L., M. Mackett, and B. Moss. Infectious vaccinia virus recombinants that express hepatitis B virus surface antigen. *Nature. 302*:490–495, 1983.

Snydman, D.R., A. Munoz, B.G. Werner, B.F. Polk, D.E. Craven, R. Platt, C. Crumpacker, R. Ouellet-Hellstrom, B.

Nash, G.F. Grady, and J.L. Dienstag. A multivariate analysis of risk factors for hepatitis B virus infection among hospital employees screened for vaccination. *Am. J. of Epidemiol. 120*:684–693, 1984.

Standring, D.N., W.J. Rutter, H.E. Varmus, and D. Ganem. Transcription of the hepatitis B surface antigen gene in cultured murine cells initiates within the pre-S region. *J. Virol. 50*, 1984.

Standring, D.N., L.B. Rall, O. Laub, and W.J. Rutter. Hepatitis B virus encodes an RNA polymerase III transcript. *Mol. Cell Biol. 3*:1774–1782, 1983.

Stevens, C.E., H.J. Alter, P.E. Taylor, E.A. Zang, E.J. Harley, and W. Szmuness. Hepatitis B vaccine in patients receiving hemodialysis. Immunogenicity and efficacy. *N. Engl. J. Med. 311*:496–501, 1984.

Stevens, C.E., W. Szmuness, A.I. Goodman, S.A. Weseley, and M. Foting. Hepatitis B. vaccine: immune responses in hemodialysis patients. *Lancet 2*:1211–1213, 1980.

Summers, J. and W.S. Mason. Properties of hepatitis B-like viruses related to their taxonomic classification. *Hepatology 2*:615–655, 1982.

Summers, J., and W.S. Mason. Replication of the genome of a hepatitis B-like virus by reverse transcription of an RNA intermediate. *Cell 29*:403–415, 1982.

Szmuness, W., C.E. Stevens, E.A. Zang, E.J. Harley, and A. Kellner. A controlled clinical trial of the efficacy of the hepatitis B vaccine (Heptavax B): a final report. *Hepatology 1*:377–385, 1981.

Szmuness, W., C.E. Stevens, W.R. Oleszko, and A. Goodman. Passive-active immunization against hepatitis B: immunogenicity studies in adult Americans. *Lancet 1*:575–577, 1981.

Szmuness, W., C.E. Stevens, E.J. Harley, E.A. Zang, H.J. Alter, P.E. Taylor, A. DeVera, G.T. Chen, and A. Kellner. Hepatitis B vaccine in medical staff of hemodialysis units: efficacy and subtype cross-protection. *N. Engl. J. Med. 307*:1481–1486, 1982.

Szmuness, W., C.E. Stevens, E.J. Harley, E.A. Zang, P.E. Taylor, and H.J. Alter. The immune response of healthy adults to a reduced dose of hepatitis B vaccine. *J. Med. Virol. 8*:123–129, 1981.

Szmuness, W., A.R. Neurath, C.E. Stevens, N. Strick, and E.J. Harley. Prevalence of hepatitis B "e" antigen and its antibody in various HBsAg carrier populations. *Am. J. Epidemiol. 113*:113–121, 1981.

Szmuness, W., C.E. Stevens, E.J. Harley, E.A. Zang, A.R. Oleszko, D.C. William, R. Sadovsky, J.M. Morrison, and A. Kellner. Hepatitis B vaccine: demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. *N. Engl. J. Med. 303*:833–841, 1980.

Tabor, E., R.H. Purcell, W.T. London, and R.J. Gerety. Use and interpretation of hepatitis B virus inocula with titered infectivity. *J. Infect. Dis. 147*:531–534, 1983.

Tabor, E., P. Snoy, D.R. Jackson, Z. Schaff, P.M. Blatt, and R.J. Gerety. Additional evidence for more than one agent of human non-A, non-B hepatitis. Transmission and passage studies in chimpanzees. *Transfusion 24*:224–230, 1984.

Tabor, E., C.R. Howard, J. Skelly, P. Snoy, A. Goudeau, A.J. Zuckerman, and R.J. Gerety. Immunogenicity in chimpanzees of experimental hepatitis B vaccine prepared from intact hepatitis B virus, purified polypeptides, or polypeptides micells. *J. Med. Virol. 10*:65–74, 1982.

Tabor, E., L.F. Barker, and R.J. Gerety. Failure to detect infectious hepatitis B virus using high dose safety test for hepatitis B vaccine. *J. Med. Virol. 6*:279–284, 1981.

Tabor, E. and R.J. Gerety. Inactivation of an agent of human non-A, non-B hepatitis by formalin. *J. Infect. Dis. 142*:767–770, 1980.

Tabor, E., D. Trichopoulos, O. Manousos, X. Zavitsanos, J.A. Drucker, and R.J. Gerety. Absence of an association between past infection with hepatitis A virus and primary hepatocellular carcinoma. *Int. J. Epidemiol. 9*:221–223, 1980.

Tabor, E., L.B. Seeff, and R.J. Gerety. Chronic non-A, non-B hepatitis carrier state: transmissible agent documented in one patient over a 6-year period. *N. Engl. J. Med. 303*:140–143, 1980.

Tabor, E., D.A. Peterson, M. April, L.B. Seeff, and R.J. Gerety. Transmission of human non-A, non-B hepatitis to chimpanzees following failure to transmit GB agent hepatitis. *J. Med. Virol. 5*:103–108, 1980.

Thung, S.N., M.S. Gerber, R.H. Purcell, W.T. London, K.B. Mihalik, and H. Popper. Animal model: chimpanzee carriers of hepatitis B virus. *Am. J. Path. 105*:328–332, 1981.

Thung, S.N. and M.A. Gerber. Polyalbumin receptors: their role in the attachment of hepatitis B virus to hepatocytes. *Semin. Liver Dis. 4*:69–75, 1984.

Thung, S.N. and M.A. Gerber. Albumin receptors on hepatitis B virus and antibodies to albumin. *Liver 1*:75–80, 1981.

Ticehurst, J.R., V.R. Racaniello, B.M. Baroudy, D. Baltimore, R.H. Purcell, and S.M. Feinstone. Molecular cloning and characterization of hepatitis A virus cDNA. *Proc. Natl. Acad. Sci. USA 80*:5885–5889, 1983.

Tyler, G.V., J. Summers, and R.L. Snyder. Woodchuck hepatitis virus in natural woodchuck populations. *J. Wildlife Dis. 17*:297–301, 1981.

Valenzuela, P., A. Medina, W.J. Rutter, G. Ammerer, and B.D. Hall. Synthesis and assembly of hepatitis B virus surface antigen in yeast. *Nature 298*:347–350, 1982.

Vernon, A.A., C. Schable, and D. Francis. A large outbreak of hepatitis A in a day-care center: association with non-toilet-trained children and persistence of IgM antibody to hepatitis A virus. *Am. J. Epidemiol. 115*:325–331, 1982.

Villarejos, V.M., K.A. Visona, and J. Bustillos. Anti-HBc development in acute and chronic type B hepatitis. *Gastroenterology 78*:1325, 1980.

Villarejos, V.M., J.C. Serra, K.A. Visona, and C.E. Eduarte. Development of chronic non-A/non-B viral hepatitis. *Gastroenterology 78*:1325, 1980.

Visona, K.A., and V.M. Villarejos. Identity of antibody to hepatitis B e/1 antigen as an atypical rheumatoid factor. *J. Infect. Dis. 141*:603–608, 1980.

Visona, K.A., and V.M. Villarejos. Antibody to hepatitis B/e antigen and rheumatoid factor. *J. Infect. Dis. 144*:292–293, 1981.

Vyas, G.N. and H. Perkins. Non-B posttransfusion hepatitis associated with hepatitis B core antibodies in donor blood. *N. Engl. J. Med. 306*:749–750, 1982.

Weiser, B., D. Ganem, C. Seeger, and H.E. Varmus. Closed circular viral DNA and asymmetric heterogeneous forms in livers from animals infected with ground squirrel hepatitis virus. *J. Virol. 48*:1–9, 1983.

Weissberg, J.E., L.L. Andres, C.I. Smith, S. Wick, J.E. Nichols, G. Garcia, W.S. Robinson, T.C. Merigan, and P.B. Gregory. Survival in chronic hepatitis B: analysis of 379 patients. *Ann. Int. Med.* (in press), 1984.

Wen, Y.-M., K. Mitamura, B. Merchant, Z.Y. Tang, and R.H. Purcell. A nuclear antigen detected in hepatoma cell lines containing integrated hepatitis B virus DNA. *Infect. Immun. 39*:1361–1367, 1983.

Wong, D.C., J.W.-K. Shih, R.H. Purcell, J.L. Gerin, and W.T. London. Natural and experimental infection of woodchucks with the woodchuck hepatitis virus as measured by new specific assays for woodchuck surface antigen and antibody. *J. Clin. Microbiol. 15*:484–490, 1982.

Xu, Z.Y., S.C. Duan, R.J. Chen, S.G. Qian, Q.R. Zhu, Z.H. Ge, S.L. Ren, H.F. Xu, Y.M. Wen, C.Y. Wang, J.X. Wange, S.L. Hu, D.M. Wang, C.B. Liu, Z.F. Liu, Z.H. Hu, Z.L. Gong, Z.X. Huang, D.P. Francis, R.H. Purcell, J. Maynard, S. Hadler, J. Gerin, B. Murphy, C. Schable, and F. Tyeryar. Prevention of hepatitis B virus carriage of infants using HBV vaccine: preliminary report of a randomized double-blind placebo-controlled trial. *Chinese J. of Infect. Dis. 8*:149–152, 1984.

Yarrish, R.L., B.G. Werner, and B.S. Blumberg. Association of hepatitis B virus infection with hepatocellular carcinoma in American patients. *Int. J. Cancer 26*:711–715, 1980.

Joint Scientific Conferences

HEPATITIS PANELS

Date: March 24–25, 1980

Place: New York, N.Y.

Summary: The sessions were devoted to discussions of hepatitis A, hepatitis B, and non-A, non-B hepatitis. The reports of studies of hepatitis A included: 1) the characterization of HAV as an agent whose genome consists of linear single stranded ribonucleic acid, 2) primary isolation of HAV in various tissue cultures, 3) development of sensitive and specific assays to detect HAV and anti-HAV, and 4) worldwide seroepidemiological surveys. The report of studies of hepatitis B included: 1) characterization of the HBV virion, 2) preparation of monoclonal antibodies to HBsAg, 3) worldwide seroepidemiological surveys in various population groups, 4) evaluation of newly developed hepatitis B vaccines, 5) therapeutic trials of various antiviral agents in patients with chronic active hepatitis B infection, and 6) prospective studies and subsequent development of cirrhosis and/or hepatoma. Additional sessions were devoted to discussions of other viruses such as woodchuck hepatitis virus and other hepadnaviruses, non-A, non-B candidate viruses, and the newly recognized delta agent.

Date: November 20–21, 1980

Place: Hakone, Kanagawa, Japan

Summary: The sessions of the second U.S.-Japan Hepatitis Research Conference were devoted to discussions of hepatitis A; hepatitis B; and non-A, non-B hepatitis. The new developments in hepatitis A research included: 1) results of seroepidemiological surveys in the United States and Nepal, 2) confirmation of the cultivation of HAV in various cell cultures, and 3) the report of the development of a hepatitis A vaccine from infected marmoset liver. Progress in hepatitis B research included: 1) propagation of defined portions of the HBV genome in cultured primate cells, 2) production of monoclonal antibodies against HBeAg in hybridoma cells, 3) a prospective study that confirmed the association between chronic hepatitis B and both cirrhosis and hepatoma, 4) the results of studies that revealed the efficacy of hepatitis B immune globulin for the prevention of hepatitis B in homosexual men, and 5) preliminary studies with such antiviral agents as human leukocyte interferon and adenine arabinoside for the treatment of chronic hepatitis B infection. The discussion of non-A, non-B hepatitis included data that confirmed the association between elevated levels of serum ALT in blood donors and the subsequent occurrence of hepatitis in recipients of the blood.

Date: March 22–23, 1982

Place: Washington, D.C.

Summary: The sessions of the Third U.S.-Japan Hepatitis Research Conference were devoted to new developments in hepatitis A; hepatitis B; and non-A, non-B hepatitis research. The discussions of hepatitis A included: 1) the results of the seroepidemiological surveys in various Asian countries and 2) the results of studies with experimental inactivated and live HAV vaccines. The hepatitis B reports included: 1) a description of the characterization and properties of the delta agent, 2) studies of the natural history of asymptomatic HBV carriers and patients with chronic hepatitis B, 3) an evaluation of the diagnostic significance of anti- HBc IgM, 4) the epidemiology of hepatitis B and hepatoma in Japan, 5) studies with woodchucks as animal models to assess the role of the HBV as a cause of hepatoma, 6) the effect of combined passive (HBIG) and active (hepatitis B vaccine) immunization for the prevention of neonatal hepatitis B infection, 7) a progress report of hepatitis B vaccine efficacy studies in renal dialysis staff and patients, and 8) current status of studies with antiviral agents for the treatment of chronic hepatitis B. The discussion of non-A, non-B hepatitis included ongoing efforts to identify and characterize non-A, non-B agents.

Date: March 9–10, 1983

Place: Kyoto, Japan

Summary: The sessions of this fourth research conference were devoted to a discussion of a wide range of topics that included 1) development of a live attenuated hepatitis A vaccine; 2) HBV DNA-a description of the genome and its expression; 3) various animal models of HBV infection, such as duck hepatitis virus and woodchuck hepatitis virus; 4) epidemiological and clinical aspects of delta virus infection; 5) the use of hepatitis B immune globulin and hepatitis B vaccine for the prevention of HBV infection of infants born to HBsAg-positive mothers; 6) treatment of chronic hepatitis B with various drugs, such as interferon, adenine arabinoside, adenine arabinoside monophosphate, cianidanol and corticosteroids; and 7) epidemiological aspects of non-A, non-B hepatitis.

Date: March 8–11, 1984

Place: San Francisco, California

Summary: The scientific portion of this meeting was held in conjunction with the International Symposium on Viral Hepatitis, from March 8–10, 1984. A business meeting of panel members was conducted on March 11, 1984. The proceedings of the International Symposium on Viral Hepatitis were published as a monograph entitled "Viral Hepatitis and Liver Disease," edited by G.N. Vyas, J.L. Dienstag, and J.H. Hoofnagle, 1984 by Grune and Stratton, Inc. As indicated in the preface of these proceedings "the meeting focused mainly on hepatitis B virus (HBV) infection. Application of technical advances in molecular and cell biology have brought about an unprecedented growth in knowledge about the biology of HBV, establishing its primary role as a cause of not only chronic liver disease but also hepatoma. Commanding less attention, but equally elegant, have been advances in hepatitis A virus (HAV) research, which have led to the cloning and sequencing of its genome and its definitive classification as an enterovirus. New information suggests that non-A, non-B hepatitis is not only very common but is also a major cause of disability and death from chronic liver disease. Despite this, the non-A, non-B hepatitis agent(s) remain elusive. In contrast, the delta agent, which was unknown just a few years ago, has been extensively characterized. The unique structure, clinical features, and epidemiology of this agent have been sufficiently defined and its designation as the hepatitis D virus (HDV) has been proposed."

LEPROSY PANEL

Drs. Masahide Abe and Robert Hastings, Chairmen respectively of the Leprosy Panel, U.S.-Japan Cooperative Medical Science Program

Dr. Masahide Abe, Chairman (1980
Member 1970-1980)
Director, National Institute for Leprosy Research
Higashimurayama-shi
Tokyo, Japan

Dr. Robert C. Hastings, Chairman (1980
Member 1977-1980)
Chief, Pharmacology Research Department
U.S. Public Health Service Hospital
Carville, Louisiana 70721

PANEL MEMBERS

Japan

Dr. Tonetaro Ito (1974–)
Professor
Department of Leprology
Research Institute for Microbial Diseases
Osaka University
Yamadakami, Suita, Osaka
Japan

Dr. Yoshiyasu Masuo (1982–)
Professor
Department of Bacteriology
Hiroshima University School of Medicine
Minami-ku
Hiroshima-shi 734, Japan

Dr. Kazunari Nakamura (1984–)
Chief
Animal Science Laboratory
National Institute for Leprosy Research
Higashimurayama-shi
Tokyo 189, Japan

United States

Dr. Patrick J. Brennan (1983–)
Associate Professor
College of Veterinary Medicine and Biomedical Sciences
Department of Microbiology
Colorado State University
Fort Collins, Colorado 80523

Dr. Thomas M. Buchanan (1980–)
Associate Professor of Medicine and Pathobiology
Immunology Research Laboratory
Pacific Medical Center
1200 - 12th Ave. South
Seattle, Washington 98144

Dr. Zanvil Cohn (1984–)
Professor and Senior Physician
Rockefeller University
1230 York Avenue
New York, New York 10021

Dr. Thomas H. Rea
Department of Health Services, Los Angeles County
USC Medical Center
1200 N. State Street, Rm. 8441
Los Angeles, CA 90033

Former Members

Dr. Takehisa Akiyama (1981-1984)
Professor
Department of Microbiology
Kitasato University
School of Medicine
1-15-1, Kitasato, Sagamihara-shi
Kanagawa Prefecture 228, Japan

Dr. Yoichiro Kawaguchi (1982-1984)
Director
2nd Research Department
National Institute for Leprosy Research
4-2-1, Aoba-cho, Higashimurayama-shi
Tokyo 189, Japan

Dr. Masahiro Nakamura (1973-1982)
Professor
Department of Bacteriology
Kurume University School of Medicine
67, Asahi-cho
Kurume-shi 830, Japan

Dr. Masashi Namba
Chairman (1974-1980)
Kyonan Hospital
1806, Kajikasawa, Minami-Kyomagun
Yamanashi Prefecture, Japan

Dr. Seitaro Okada (1978-1982)
Director
National Sanatoria for Oshima-Seishoen
6034-1, Anji-machi, Kita-gun
Kagawa Prefecture 761-01, Japan

Former Members

Dr. Barry Bloom, Chairman (1977-1980,
Member 1975-1977)
Professor
Microbiology, Immunology and
Cell Biology
Albert Einstein College of Medicine
Bronx, New York 10461

Dr. Ward E. Bullock (1983-1984) Resigned
Director, Division of Parasitic Diseases
University of Cincinnati
College of Medicine
231 Bethesda Avenue
Room 7168
Cincinnati, Ohio 45267

Dr. A. Howard Fieldsteel (1977-1982)
Deceased
Manager, Infectious Diseases Program
Stanford Research Institute
333 Ravenswood Avenue
Menlo Park, California 94025

Dr. Wayne M. Meyers (1977-1983)
Chief, Microbiology Division
Department of Infectious and
P.O. Pathology
Armed Forces Institute of Pathology
6825 16th Street, N.W.
Washington, D.C. 20306

Japanese Leprosy Panel

BROAD GOALS

The chemotherapy of leprosy is fraught with problems such as the appearance of drug-resistant bacilli, relapse caused by persistant organisms, and functional disabilities due to adverse reactions. The development of an effective vaccine for the prophylaxis of leprosy requires additional research. These problems should be overcome by promoting research in the following four areas which have been pursued without substantial change over the last two decades:

1. Microbiology of *M. leprae*: Since *in vitro* cultivation of *M. lepraemurium* has been accomplished, these findings will be applied to the cultivation of *M. leprae*. Information about biochemical properties of this bacillus will be useful in cultivation studies and other phases of research.
2. Experimental leprosy: A lepromatous leprosy model has been established in nude mice. This animal model is used for chemotherapy and immunopathology studies of leprosy and also serves as a source of *M. leprae* bacilli. To increase the sensitivity of this model, it will be necessary to use nude mice that are highly susceptible to *M. leprae*.
3. Chemotherapy of leprosy: The effectiveness of antileprosy drugs and the mechanisms of their action have been investigated using the nude mouse leprosy model. This model will be useful in the development of new drugs for leprosy. The study will be accelerated by the introduction of modern analytical and pharmacological methods. Studies on immunotherapy should be pursued as an adjunct to chemotherapy.
4. Immunology of leprosy: Serological tests specific for detecting *M. leprae* have been developed and used for diagnosing primary leprosy infections and for detecting subclinical cases. The mechanism of the immune response in leprosy was investigated at the cellular level and immunogenetically. The results of these studies will be useful in the development of immunotherapy and protective vaccines.

PROGRESS IN PRIORITY AREAS

Microbiology of *M. Leprae*

Progress has been noted in studies concerning the biochemistry of *M. leprae* and in cultivation studies of *M. lepraemurium*. Major contributions are as follows:

Purification of *M. leprae*: Density gradient centrifugation methods were used for purifying *M. leprae* from an infected armadillo liver using Percoll and from nude mouse footpad using Ficoll 400. The purified bacilli were obtained in pure form without enzymatic action which reduced the processing time. The procedure did not constitute a biological hazard to the investigators.

The respiratory system of *M. leprae*: The number of carbons attached to alkyl side chains of alpha-mycolic acid of *M. leprae* was 20, which was 2 to 4 less than those of other mycobacteria. This finding will be useful in identifying *M. leprae*. The $^{C}22{:}0/^{C}24{:}0$ value among the saturated fatty acids of *M. leprae* was higher than those of other mycobacteria. Since this assay can be done simply with few organisms, the procedure could be applied to tentatively differentiate *M. leprae* in an unknown specimen. In addition to the alpha-mycolic acid, *M. leprae* has a mycolic acid which is similar to gamma-mycolic acid of *M. tuberculosis* but apparently specific for *M. leprae*.

Substances surrounding *M. leprae* cells: Acetone-soluble lipids of armadillo lepromas were analyzed. The lipid with a molecular weight of approximately 2,000 corresponded to phenol glycolipid I of Brennan's; the lipid with the molecular weight of 1,100 contains no phenol and was the same as phthiocerol dimycocerosate from *M. leprae*; and another lipid with the molecular weight of 900 was detected, and this lipid is found in normal mammalian tissues.

Cultivation: Progress has been made in cultivating *M. lepraemurium* in a cell-free liquid medium and in a cell culture system. However, there is no evidence that *M. leprae* multiplies in any of these systems. Multiplication of *M. lepraemurium* in the cell-free liquid medium was accelerated by the addition of liposome, egg yolk, dextran, vitamins, and others, and continuous growth was maintained throughout 3 to 4 successive cultures. In the cell culture system of *M. lepraemurium*, the balance between the growth rate of the organism and that of the host cell was controlled by adding cycloheximide to the culture medium. With this procedure the cell culture system gave reproducible results. Such data should contribute to the cultivation studies of *M. leprae*.

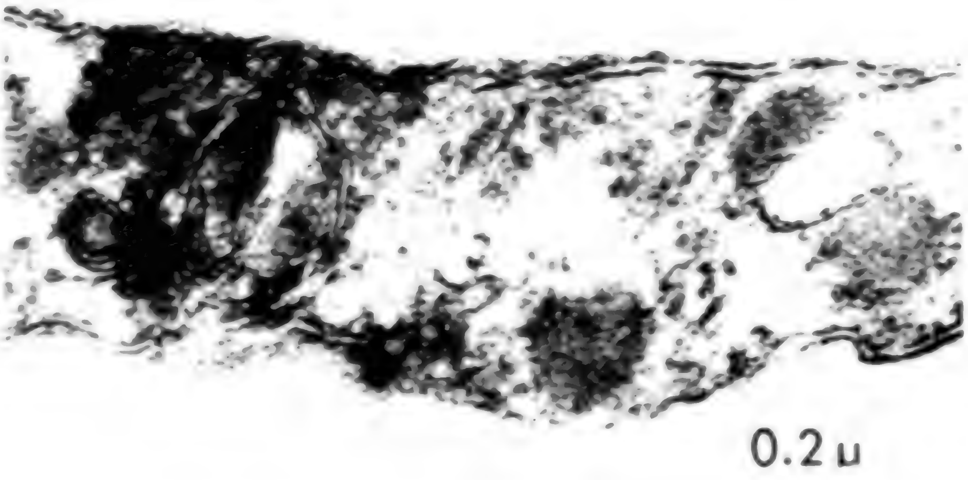
Experimental Leprosy

Investigators searched for animals more susceptible to *M. leprae* infection than athymic rodents, but such an animal was not found among the rhinomouse, congenital asplenic mouse, molossinus mouse, *Suncus murinus*, *Micromys minutus*, and hairless *(ha)* rat.

The treatment of Rowett nude (*rnu*) rats infected with *M. leprae* with carrageenan and with thymus cells was carried out. In the carrageenan pretreated rat, marked multiple nodular swellings appeared on the infected limb, mouth, face, ears, lumbar sites, tail to external genitalia skin, and on the uninoculated fore and hind limbs. In the group pretreated with thymus cells, bacillary counts were reduced 10 to 100 fold when compared to the control animals.

Chemotherapy and immunotherapy trials in nude mice and nude rats were investigated and compared to those of *M. avium* and *M. lepraemurium* infections using various strains of nude mice.

Multiple inoculation sites such as, the extremities (e.g., lower legs and feet), upper lips, dorsal-lumbar sites, i.p., perianal skin (base of tail to external genitalia), and both testicles, produced



Intracytoplasmic membrane systems of *Mycobacterium leprae*. Continuity of the cytoplasmic membrane with the intracytoplasmic membranous organelle is distinguishable.

matic lesions when compared to a simple inoculation site.

In comparing BALB/c nu, C57BL/6 nu, C57BL nu, NC nu, NZB nu, AKR/J nu, C3H/HeN (MTV (having murine tumor virus), CBA/N nu, NFS/N nu, NIH(S) nu, and ICR nu mice for susceptibility to the growth of *M. leprae*, the NFS/N nu and N:NIH(S) nu mice were confirmed to be a powerful tool as an experimental leprosy model when the upper lip inoculation was used. In the case of hind foot infection, C3H/HeN nu and CBA/N nu mice developed heavier infection with necrosis than NFS/N nu and N:NIH(S) nu mice. Therefore, the development of heavy lepromatoid lesions in the nude mice was markedly influenced by the genetic background of the animals. Moreover, the formation of lepromatoid lesions in the nude mouse was enhanced by the combination with other genes such as the hereditarily asplenic athymic (LASAT) mice, C57BL/6 bg nu and C57BL/6J bg nu mice, as compared to same genetic background of nude mice. Additionally, in comparing SHR rnu, ACI rnu, LOU/CN rnu, WM rnu, and LEW rnu rats for susceptibility to growth of *M. leprae*, the SHR nude rat gave excellent results with the development of a heavy lepromatoid formation. The development of a heavy lepromatoid formation in nude rats was markedly influenced by the genetic background of the rats, which simulated the results with nude mice. Blood pressure among the various nude rats corresponded to those strains with hereditary hypertension, except for the WM rnu rat. Therefore, athymic rodents such as NFS/N nu mice and SHR nude rats will be useful for the various studies on leprosy.

Chemotherapy of Leprosy

Blood level concentrations of anti-leprosy drugs have been obtained. Recent progress on simultaneous analysis of sulfone (DDS), rifampicin and lamprene, and/or simultaneous analysis of DDS and its metabolites by high pressure liquid chromatography was considered a useful method for study of the metabolism and pharmacology of anti-leprosy drugs.

The nude mouse became a model for studying the treatment of multibacillary leprosy with various drugs. Rifampicin produced rapid killing of *M. leprae*, but there was no effect with clindamycin, minocycline, and INAH when tested in the nude mouse. On the other hand DDS did not cause the rapid initial killing of *M. leprae* as was found with rifampicin. The unexpected results obtained with DDS led to the study of the metabolism of the drug. However, there was no significant difference in the metabolism of DDS in the nude mouse when compared to a normal mouse. It was also shown that the routine dose of DDS did not show a significant effect on the cellular immune response (CMI) in the nude mouse.

The effect of several immuno-potentiators and immunomodulators on the chemotherapy of leprosy were examined. The data indicated that diethyldithio-carbamate (DDC) had an immunomodulative effect and suppressed the growth of *M. leprae* in the mouse footpad. It appears that DDC might be a drug similar to lamprene which can control erythema nodosum leprosum (ENL) and kill *M. leprae* simultaneously. The administration of platonin to thymectomized guinea pigs produced increased rosette formation like lamprene, suggesting that platonin might be effective in controlling ENL.

Studies on experimental ENL in the nude mouse were initiated. Acute vasculitis was observed in *M. leprae* infected nude mouse footpads, and this acute vasculitis was enhanced by administering DDS and/or DDS plus rifampicin. The data indicate that the experimental ENL observed in the *M. leprae* infected nude mouse might have been caused by the administration of DDS.

The antimycobacterial effect of the extract of Sasa (Sasa Veitchi Rehd. Sasa Nipponica etc.) was examined. The extract suppressed the growth of BCG *in vitro* and the development of murine leprosy *in vivo*. Additional studies are needed before the extract may be clinically tested.

Immunology of Leprosy

Immuno-genetic factors regulating the host susceptibility to leprosy

It is universally known that CBA/J mice are very susceptible to murine leprosy, while C57BL/6 mice show a relative resistance to the infection. An investigator of our panel attempted to elucidate the difference in susceptibility in the above mentioned strains of mice in terms of a functional disparity of their splenic macrophages, and indicated that the difference in their phagocytic as well as digestive activities against

M. lepraemurium is involved in the phenotypic difference of susceptibility.

On the other hand, labeled DNA from *in vitro* cultured *M. lepraemurium* was tested for its basic sequence homology to cold DNA from the liver cells of the above mentioned two strains and six other strains of mice with distinct susceptibility to murine leprosy. Results showed the homology between the microbial DNA and DNA from susceptible strains of mice was shown to be greater than that between the same *M. lepraemurium* DNA and DNA from resistant strains. This suggests that the susceptibility of animals to infections due to intracellular bacteria is predetermined genetically by the degree of relatedness between DNA from microbes and hosts.

Aside from these investigations, genetic control over the clinical course of leprosy was tested using the HLA-typing of leprosy patients. It was reported that HLA-DR2 and MT-1 were significantly increased in number in unrelated patients suffering from tuberculoid leprosy in comparison with those in normal control populations. However, HLA-DR2 alone was more frequently found in the patients with lepromatous leprosy than that of controls.

Serodiagnosis of leprous patients and their contacts

It was shown that phenolic glycolipid-1 is a specific seroreactive antigenic compound from *M. leprae* infected armadillo and human tissues. Several laboratories have used this compound as an antigen for the serodiagnosis of leprosy. The most widely used method for this purpose is the enzyme-linked immunosorbent assay (ELISA), despite its several faults involving complicated manipulations and poor reproducibility. In order to overcome those shortcomings, a new qualitative complement fixation test was developed, and there was good correlation of the data between the two tests.

In addition, the actual determinant has been chemically synthesized and conjugated to linker arms; these compounds have been tested for serologic activity.

Detection of subclinical leprosy in individuals with low or no resistance against *M. leprae*, followed by treatment before overt symptoms occur, is urgently needed for control of this disease. The fluorescent antibody adsorption test is very sensitive and specific for diagnosing leprosy. This test is useful for detecting subclinical leprosy in household contacts and school children who are asymptomatic for leprosy. However, the test may be too time-consuming for a large scale survey. Possibly the lepromin test should be used for screening susceptible individuals. Preliminary information indicates that saliva may be used instead of serum for the screening test. However, this procedure needs to be studied further.

Development of an effective vaccine against leprosy

The results of the tuberculosis vaccine (BCG) field trials in India were disappointing. However, there can be a degree of optimism, for a leprosy vaccine reports that killed *M. leprae* are more effective in stimulating immunity than live *M. leprae*. It is known that cellular immunity is more important than humoral immunity in resistance to infection due to intracellular bacteria. In experimental murine typhoid, cellular immunity is induced only by live vaccines. Killed vaccines administered in conjunction with complete adjuvant induced a state of DTH to the microbial agents and were not protective against virulent challenge in the experimental infection.

ACHIEVEMENTS OF THE LEPROSY PANEL DURING THE PAST 20 YEARS

Cultivation of *M. Leprae*

The most significant advance made by the Japanese scientists during the past 2 decades was the establishment of methods for multiplication of *M. lepraemurium* on solid medium and in cell-free liquid medium. This resulted in extensive progress in the biochemistry of this organism and stimulated the study of cultivation of *M. leprae*. The supply of large numbers of *M. leprae* cells from the infected armadillos and nude mice provided marked progress in studies on biochemistry, especially cellular components on *M. leprae*. The nude mouse is now an indispensable source of *M. leprae* for *in vitro* cultivation studies.

Experimental Leprosy

Several attempts at immunosuppression of the normal mouse did not succeed in the establishment of systemic *M. leprae* infection.

A new model of experimental lepromatous leprosy in athymic (nude) mice was established by Japanese scientists beginning in 1976. This leprosy model has been used to study experimental chemotherapy, and to compare *M. leprae* with other mycobacterial infections in nude mice.

Thymus cell grafting into C57BL/6 nude mice should have an enhancing effect on progressive leprous lesions in the treated animal as compared to the untreated C57BL/6 and BALB/c nude mice. Lepromatoid lesions in the foot of the C57BL/6 nude mouse were less severe than that of the BALB/c nude mouse. Moreover, the gnotobiotic BALB/c nude mouse showed a slightly enhanced swelling of the foot at a later stage of *M. leprae* infection. It was therefore suggested that the growth of *M. leprae* in the nude mouse might be influenced by genetic and environmental factors.

Chemotherapy of Leprosy

Significant developments in the chemotherapy of leprosy were made in the past 20 years. Recently the World Health Organization published recommendations on chemotherapy of leprosy. They were combination therapy using DDS and rifampicin for 6 months for the pauci-bacillary type of leprosy, and triple therapy with DDS, rifampicin and lamprene for 2 years for multi-bacillary type of leprosy. The U.S.-Japan Cooperative Medical Science Program's Leprosy Panel made a significant contribution to the formulation of the above-mentioned regimens. Japanese researchers have also made a significant contribution toward proving the initial killing effect of rifampicin against *M. leprae* by means of experimental leprosy in the nude mouse. The therapy of leprosy will be further promoted by developing new methods for control of ENL and the discovery on new anti-leprosy drugs.

A new era of leprosy control was derived from initiation of multi-drug therapy. In many developing countries multi-drug therapy will be applied to rapidly control leprosy. All newly detected leprosy patients in Japan can now be treated with multi-drug therapy and not require hospitalization. The development of improved chemotherapy for leprosy will change the social aspects of this disease.

Immunology of Leprosy

Antigenic specificity of *M. leprae* was demonstrated by immunodiffusion and immunofluorescence. Detection of *M. leprae*-specific antibodies was useful for the serodiagnosis of leprosy. Serological tests with synthetic antigens containing specific epitopes were also attempted. It was found that both suppressor T cells and macrophages participated in the deficient cell-mediated immunity in lepromatous leprosy. Histopathological and serological studies were conducted on adverse reactions which caused tissue damage as a result of treatment of leprosy. These immune responses in leprosy patients appeared to be controlled by immunogenetic factors, as suggested by the association of HLA-DR antigens with leprosy. Differences in immune responsiveness of mice with murine leprosy according to genetically different strains were investigated at cellular and molecular levels. These investigations are useful for the elucidation of the immunological mechanism in leprosy.

FUTURE GOALS

The success in cultivation of *M. lepraemurium* in cell-free media and in tissue culture cells, and the biochemical studies on *M. leprae* should initiate important information useful for the cultivation of *M. leprae*. Further biochemical studies will be conducted to analyze specific cellular components of *M. leprae* and/or the surrounding substances of *M. leprae* cells. These studies could help improve methods for identifying the leprosy bacilli and development of effective vaccines.

The formation of lepromatoid lesions in athymic rodents may be influenced by various factors in the endogenous and/or exogenous environments, as well as the genetic background of the animals. Therefore, the relationship among human leprosy bacilli, host, and environment should be studied. New animal models of experimental tuberculoid leprosy should be developed. The model will be compared with those in the athymic rodents.

Further efforts should be made to discover new anti-leprosy drugs for improvement of multi-drug therapy. An alternative drug for DDS is urgently needed to treat DDS resistant cases. Studies on the method to control ENL should also be expanded. Combined therapy using chemotherapy and immunotherapy should be considered in the near future.

The immunological mechanism of susceptibility and resistance against *M. leprae* should be elucidated. There is an urgent need to develop a serodiagnostic test for early detection of leprosy patients and their contacts with a high risk of infection. Fundamental studies on the superiority or inferiority of attenuated or killed vaccines, as well as on the application of gene technology, will be needed for the development of an effective vaccine for prophylaxis and therapy of leprosy.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
CULTIVATION OF *M. LEPRAE*	
Seiko Fukunishi National Leprosarium Ohshima Seisho-en	Biochemical studies on lipid components of mycobacteria, especially of *M. leprae* and peribacillary substance—using liquid chromatography—using mass spectrometry
	Affinity of *M. leprae* to peripheral nerves, in association with phenolic glycolipid and phthiocerol dimycocerosate
Tsunehiko Hirata National Institute for Leprosy Research	Ultrafine structure of *M. leprae* and *M. lepraemurium*, especially study on intra-bacterial inclusion
Yuichi Koseki National Institute of Health	Cultivation of *M. leprae* by solid medium
Takashi Kusaka Kawasaki Medical College	Biosynthesis of mycolic acid of *M. lepraemurium* and *M. smegmatis*
	Re-examination of biosynthetic activity of *M. lepraemurium*
	Study on mycolic acid of *M. leprae*
	Analysis of mycolic acid of *M. leprae*
Tyoku Matsuhashi Tokyo University	Study on sugar metabolism of *M. leprae* by nucleo-magnetic resonance

Investigator and Institution	*Title*
Yoshiyasu Matsuo Hiroshima University	Cultivation of *M. leprae* and *M. lepraemurium*
	Cell-culture of *M. leprae* and *M. lepraemurium*
	Cultivation of *M. leprae* and *M. lepraemurium* by cycloheximide-treated cells
	Differentiation of viable and non-viable *M. leprae* by fluorescent staining
Tatsuo Mori Osaka University	Cultivation of *M. leprae*
	Clarification of the cause of slow-growing mycobacteria
	Improvement of collecting method of *M. leprae*
	Cytochrome and respiratory enzyme system of *M. leprae*
	Glutamate-, ornithine- and lysine-decarboxylase activities in *M. leprae*
	Catalase, peroxydase and superoxide dismutase of *M. leprae*
Hiroko Nakagawa National Institute for Leprosy Research	Study on lipid metabolism of *M. lepraemurium*
	Studies on the membrane-bound enzymes for phospholipid metabolism in *in vivo* grown mycobacteria
Masahiro Nakamura Kurume University	Cultivation of *M. leprae* by cell-free liquid medium
	Biochemical properties of *M. lepraemurium* grown in liquid medium and their contribution to cultivation of *M. leprae*
	Cell-free cultivation of *M. leprae*—selection of culture medium using ATP as an indicator
Tetsu Nakayama National Institute for Leprosy Research	Study on cultivation of *M. leprae*
	Studies on cultivation, purification and identification of *M. leprae*
Hiroko Nomaguchi Osaka University	Cell culture of *M. leprae*
Tatsuji Ogawa Kitasato Institute	Study on cultivation of *M. leprae*

ANIMAL TRANSMISSION

Yoichiro Kawaguchi National Institute for Leprosy Research	Development of experimental leprosy by nude mice
Kenji Kohsaka Osaka University	Animal transmission of *M. leprae*—development of experimental leprosy
Masanori Matsuoka National Institute for Leprosy Research	Establishment of leprosy model in mice (rise and fall of bacterial count in KK-nude mouse and observation of host-response in conventional mouse as an indicator of cell-mediated immunity)
Kazunari Nakamura National Institute for Leprosy Research	Studies on the development of experimental leprosy—using athymic (nude) rat—immunobiological influence on lepromatous leprosy lesion in congenitally athymic (nude) animals

ANTI-LEPROSY DRUGS

Investigator and Institution	*Title*
Masaichi Gidoh National Institute for Leprosy Research	Establishment of simultaneous analysis acid-labile metabolites of DDS by high-performance liquid chromatography (HPLC).
	Analytical study on anti-leprosy drugs.
Kiyoshi Harada National Leprosarium Tama Zensho-en	Histological classification and bacteriological examination of leprosy by periodic acid-carbol-pararosaniline and periodic acid-MS methods (comparison with Z.N. method).
	Localization of *M. leprae* in leprosy lesion.
	Pathology of skin lesion in leprosy patient, especially its relation to terminal branch of peripheral nerve.
Tonetaro Ito Osaka University	Study on the mechanism of action of DDS.
	Development and evaluation of anti-leprosy drugs.
	Metabolism of DDS in nude mouse.
	Verification of the effect of anti-leprosy drugs, especially of INAH.
	Study on provocation of ENL by anti-leprosy drug.
Goyo Koya Hirosaki University	Amyloid precipitation of autonomic nerve in leprosy.
Ei ji Kubo National Tokushima Sanatorium	Determination of blood level of anti-leprosy drugs.
Masashi Namba National Institute for Leprosy Research	Therapy of cranial nerve involvement in leprosy.
Seitaro Okada Kyoto University	Fundamental study on the *in vitro* test for the effectiveness of chemical drugs.
Yoshitsugu Satake Nishikyushu University	Basic research of anti-leprosy drugs—growth-inhibitory effect of bamboo grass-extract against mycobacteria, especially against BCG.
	Study on "Leigonteng" that is said to be effective to inhibit ENL.
Sadae Tsutsumi National Institute for Leprosy Research	Development of immuno-stimulative anti-inflammatory drugs for leprosy.
	Study on development of anti-leprosy drugs.
	Study on the mechanism of action of anti-leprosy drugs—pharmacological study.
	Fundamental study on the improvement of chemotherapy of leprosy.

IMMUNOLOGY OF LEPROSY

Masahide Abe National Institute for Leprosy Research	Immunological study on subclinical leprosy infection.
Takehisa Akiyama Kitasato University	Analysis of induction of cellular immunodeficiency in mouse infected with large doses of *M. lepraemurium*.

Investigator and Institution	*Title*
	Molecular biological study on host-parasite relationship in leprosy—association with immunity.
Tsuyoshi Fujiwara Nara Medical College	Chemical synthesis of pseudoantigen specific for *M. leprae*.
Seiichi Izaki Iwate Medical College	Experimental study on granulomatous inflammation against mycobacteria by using murine leprosy.
Shinzo Izumi Kyoto University	Analysis of cellular mechanism of human immune response against *M. leprae*.
	Cellular immune responsiveness in different strains of mice against *M. leprae*.
	Possibility of early serodiagnosis of leprosy using phenolic glycolipid antigen specific for *M. leprae*.
Fuminori Kanetsuna Kyoto University	Effect of free fatty acid on mycobacteria.
Eiichi Matsuo Kyorin University	Immuno-histochemical and pathological analyses of hydrolase of cells in human lepromatous and tuberculoid granulomas.
	Immuno-histochemical analysis of β-glucuronidase and lysozyme of cells in human lepromatous and tuberculoid granulomas and renal β-glucuronidase in leprosy patient.
	Enzyme-histological anomaly in skin lesion of leprosy patient, especially the effect of abnormal β-glucuronidase on renal lesion.
	Immunological and immuno-histochemical studies on host-derived or mycobacteria-derived β-glucuronidase in human leprosy lesion.
Yasuhiro Minauchi National Leprosarium Hoshizuka Keiai-en	Study on auto-immune mechanism of peripheral nerve involvement in leprosy.
	Immuno-histochemical examination of leprosy neuritis.
Yasuji Nakajima National Leprosarium Kuriu Rakusen-en	Study on immunotherapy of leprosy.
Susumu Ohkawa Kyoto University	Humoral factor regulating cell-mediated immunity in leprosy.
Toshiharu Ozawa National Institute for Leprosy Research	Suppressor cells in lepromatous leprosy.
	Immunogenetic study on leprosy, especially on HLA-DR antigens.
	Clinico-immunological study on leprosy—assocation between HLA-DR antigen system and cell-mediated immunity (analysis of immune responses against *M. leprae* antigen).
Nanako Saito National Institute for Leprosy Research	Role of suppressor T cell in mouse infected with *M. lepraemurium*.
	Role of T cell and antigen presenting cell in mouse infected with *M. lepraemurium*.
	Macrophage function in mouse infected with *M. lepraemurium*.

Investigator and Institution	*Title*
	Genetic analysis by the interaction of T cell and macrophage of the mouse infected with *M lepraemurium*.
Yoshimoto Sakamoto National Institute for Leprosy Research	Study on drug allergy in therapy in leprosy.
	Radioimmunoassay of antibody activity against particular mycobacterial antigen in leprosy.
Hideyuki Suzuki National Leprosarium Tama Zensho-en	Immunological study on peripheral nerve damage in leprosy.
	Vasculitis and immune complex in leprosy—immunohistochemical study.
	Microvascular damage and immune complex in leprosy.

BIBLIOGRAPHY

Abe, M. , F. Minagawa, Y. Yoshino, T. Ozawa, K. Saikawa, and T. Saito. Fluorescent leprosy antibody absorption (FLA-ABS) test for detecting subclinical infection with *Mycobacterium leprae*. *Int. J. Lepr. 48*, 109–119, 1980.

Abe, M., T. Ozawa, F. Minagawa, and Y. Yoshino. Subclinical infection in leprosy—its detection and control by fluorescent leprosy antibody absorption (FLA-ABS) test. *Lepr. Rev. 52* (Suppl. 1), 263–273, 1981.

Abe, M. Subclinical infection in leprosy—Detection and control by immunofluorescence (FLA-ABS) test. *MODAN MEDIA* 27, 241–250, 1981 (in Japanese).

Abe, M. Some problems on leprosy vaccine. *Jap. J. Bact. 38* (Suppl.), 89–91, 1983 (in Japanese).

Abe, M. Serodiagnosis of Hanseniasis by indirect immunofluorescence. Proceedings of the XVIth International Congress of Dermatology (Kukita, A. and Seiji, M. ed.), University of Tokyo Press, Tokyo, pp. 459–462, 1983.

Abe, M. Fundamental and practical requirements of serological test for detecting subclinical infection in leprosy. Proceedings of the Workshop on Serological Tests for Detecting Subclinical Infection in Leprosy. Sasakawa Memorial Health Foundation, Tokyo, pp. 5–18, 1983.

Abe, M. The lepromin reaction as an indicator of cell-mediated immunity in leprosy. Progress in Immunology of Leprosy (Talwar, G.P., J.L. Turk, and R.J.W. Rees, ed.), Arnold-Heinemann Publishers, New Delhi, pp. 138–147, 1983.

Abe, M., Y. Yoshino, F. Minagawa, I. Miyaji, P. Sampoonachot, T. Ozawa, Y. Sakamoto, T. Saito, and K. Saikawa. Salivary immunoglobulins and antibody activities in leprosy. *Int. J. Lepr. 52*, 343–350, 1984.

Akiyama, T. Molecular biology of host-parasite relationship in infectious diseases, especially in salmonellosis, tuberculosis and leprosy. *Microbiol.-1984*, 355–362, 1984.

Cho, S. N., T. Fujiwara, S.W. Hunter, T.H. Rea, R.H. Gelber, and P.J. Brennan. Use of an artificial antigen containing the 3,6-di-*O*-methyl-*β*-D-glucopyranosyl epitope for the serodiagnosis of leprosy. *J. Infect. Diseases 150*, 311–322, 1984.

Colston, J., and K. Kohsaka. The nude mouse in studies of leprosy. The Nude Mouse in Experimental and Clinical Research, 2, pp. 247–267, Academic Press, New York, 1982.

Esashika, I., N. Osawa, T. Mori, and T. Akiyama. Molecular biology of host-parasite relationship in infectious diseases. V. DNA homology between two bacterial strains of *M. lepraemurium* and *S. enteritidis* and several inbred strains of mice with different susceptibility to the infections due to the respective bacilli. *Kitasato Med. 14*, 95–101, 1984 (in Japanese).

Fujiwara, T., S.W. Hunter, S-N. Cho, G.O. Adpinall, and P.J. Brennan. Chemical synthesis and serology of the di- and trisaccharides of the phenolic glycolipid antigens from the leprosy bacillus and preparation of a disaccharide protein conjugate for serodiagnosis of leprosy. *Infect. Immun. 43*, 245–252, 1984.

Fukunishi, Y., S. Okada, M. Nishiura, and K. Kohsaka. Study of experimental leprosy in nude mice inoculated with *M. leprae*-light and electron microscopic findings. *Jap. J. Lepr. 50*, 192–203, 1981 (text in Japanese).

Fukunishi, Y., S. Okada, M. Nishiura, and K. Kohsaka. Ultrastructural feature of the multiplication of human and murine leprosy bacilli in macrophages of nude mice. *Int. J. Lepr. 50*, 68–75, 1982.

Fukunishi, Y., W.M. Meyers, G.P. Walsh, F.B. Johnson, C.H. Binford, S. Okada and M. Nishiura. Ultrastructural features of macrophages of armadillos infected with actively multiplying *Mycobacterium leprae*. *Int. J. Lepr. 52*, 198–202, 1984.

Fukunishi, Y., W.M. Meyers, C.H. Binford, G.P. Walsh, F.B. Johnson, P.J. Gerone, R.H. Wolf, B.J. Gormus, and L.N. Martin. Electron microscopic study of leprosy in a mangabey monkey (natural infection). *Int. J. Lepr. 52*, 203–207, 1984.

Gidoh, M., and S. Tsutsumi. On the immunodepressive effect of Rifampicin on cell-mediated immunity of guinea pigs, examined by rabbit red blood cell rosette formation method. *Jap. J. Lepr. 50*, 55-61, 1981.

Gidoh, M., S. Tsutsumi, and S. Takitani. Determination of three main antileprosy drugs and their main metabolites in serum by high-performance liquid chromatography. *J. Chromatogr. 223*, 379–392, 1981.

Gidoh, M., and S. Tsutsumi. Studies on the derivatives showing local anesthetic actions of several acidic antiinflammatory drugs, aiming at the possibility of treatment for leprous neuritis. *Jap. J. Lepr. 52*, 156–164, 1983.

Gidoh, M., and S. Tsutsumi. Studies on search for a promising immunopotentiative substance for treatment of leprosy. III. With special reference to an immunomodurative drug CCA, a BCG vaccine and the significance of an improved rabbit red blood cell rosette formation test. *Jap. J. Lepr. 52*, 165–179, 1983.

Gidoh, M., S. Tsutsumi, and S. Kamohara. Studies on search for a promising immunopotentiative substance for treatment of leprosy. IV. On several synthesized amphoteric dextrans exhibiting both immunopotentiative and anti-exudative actions. *Jap. J. Lepr. 52*, 200–216, 1983.

Gidoh, M., S. Tsutsumi, S. Kaniwa, M. Narita, J. Matsumura, S. Takitani, and K. Fukushi. Studies on search for a promising immunopotentiative substance for treatment of leprosy. I. Analytical studies on bamboo grass. *Jap. J. Lepr. 49*, 38–46, 1980.

Gidoh, M., S. Tsutsumi, S. Kaniwa, M. Narita, J. Matsumura, S. Takitani, K. Fukushi, and H. Satoh. Studies on search for a promising immunopotentiative substance for treatment of leprosy. On several biological actions of bamboo grass extract. *Jap. J. Lepr. 49*, 47–53, 1980.

Hibino, T., S. Izaki, and M. Izaki. Detection of serine proteinase inhibitors in human cornified cells. *Biochem. Biophys. Res. Commun. 101*, 948–955, 1981.

Hibino, T., S. Izaki, Y. Isozaki, H. Shimoda, P.S. Hsu, and M. Izaki. Plasminogen activator (PA) and PA inhibitor associated with granulomatous inflammation. *Blood and Vessel 14*, in press, 1984 (in Japanese).

Hirata, T. Electron microscopic observations of intracytoplasmic inclusions in human and murine leprosy bacilli. *Int. J. Lepr. 51*, 84–88, 1983.

Hirata, T. Electron micrographs of murine leproma. *Jap. J. Lepr. 52*, 65–72, 1983 (in Japanese).

Hirooka, Y., and N. Saito. The strain difference of immune response in mice infected with *Mycobacterium lepraemurium*. *Jap. J. Lepr. 50*, 144–153, 1981.

Hunter, S.W., T. Fujiwara, and P.J. Brennan. Structure and antigenecity of the major specific glycolipid antigen of *Mycobacterium leprae*. *J. Biol. Chem. 257*, 15072–15078, 1983.

Ito, T. Treatment of leprosy and results obtained by the treatment. *SOGORINSHO 30*, 1602–1606, 1981 (in Japanese).

Ito, T. Anti-leprosy drugs. *SOGORINSHO 30* (Suppl.), 781–782, 1981 (in Japanese).

Izaki, S., W.L. Epstein, and K. Fukuyama. Tissue proteinase inhibitor in epithelioid cell granuloma. *In*: Sarcoidosis and Other Granulomatous Diseases. (Williams, W.J. and B.H. Davis, ed.), Alpha Omega Pub., Cardiff, Wales, pp. 172–176, 1980.

Izaki, S., S.M. Goldstein, K. Fukuyama, and W.L. Epstein. Tissue induction of a urokinase inhibitor associated with chronic granulomatous inflammation: correlation with local fibrin deposition and clearing. *Blood and Vessel 11*:675–678, 1980 (in Japanese).

Izaki, S., Y. Isozaki, T. Hibino, M. Satoh, S. Kon, and M. Izaki. Immune reaction and tissue plasminogen activator (PA): A preliminary report on studies with murine leprosy developing in two mouse strains with diverse immunoreactivity. *Blood and Vessel 13*:351–354, 1982 (in Japanese).

Izaki, S., Y. Isozaki, M. Satoh, T. Hibino, S. Kon, and M. Izaki. Comparative study with two polar types of murine leprosy: An involvement of plasminogen activator and its possible regulating factor in the granulomatous tissue reaction. *J. Inv. Dermatol. 80*:81–85, 1983.

Izaki, S., T. Hibino, Y. Isozaki, P.S. Hsu, M. Izaki, and O. Matsuo. Plasminogen activator and plasminogen activator inhibitor associated with granulomatous inflammation: A study with murine leprosy. Thromb. *Haemostas. 52*; in press, 1984.

Izaki, S., T. Hibino, H. Shimoda, and M. Izaki. Plasminogen activator in granulomatous inflammation. In: Chemical Mediators of Inflammation. (S. Cohen, H. Hayashi, K. Saito, A. Takada, eds.), Academic Press, New York, in press.

Izaki, S., T. Hibino, H. Shimoda, P.S. Hsu, O. Tanji, S. Tokairin, M. Ohkuma, M. Izaki, and O. Matsuo. Murine leprosy: An animal model to investigate proteinase-proteinase inhibitor regulation system in inflammation. In: The Biological Role of Proteinase and Their Inhibitors in Skin. (Ogawa, H. and Lazarus, G.S. eds.), Tokyo University Press, Tokyo, in press.

Kashiwabara, Y., H. Nakagawa, and G. Matsuki. Phospholipid deacylating activities in murine leprosy bacilli. *J. Biochem. 88*:1861–1868, 1980.

Kato, H., K. Sanada, M. Koseki, and T. Ozawa. Identification of lymphocyte subpopulation in cutaneous lesions of leprosy. *Jap. J. Lepr.* 52:126–132, 1983 (in Japanese).

Kawaguchi, Y., M. Matsuoka, K. Sushida, and M. Tanemura. Susceptibility to murine leprosy bacilli of C3H/He mice. *Jap. J. Lepr. 49*:14–19, 1980 (in Japanese).

Kawaguchi, Y., M. Matsuoka, K. Sushida, and M. Tanemura. Susceptibility to *Mycobacterium avium* of C3H/He mice. *Jap. J. Lepr. 50*:128–134, 1981 (in Japanese).

Kawaguchi, Y., M. Matsuoka, K. Sushida, and M. Tanemura. Susceptibility to *Mycobacterium avium* of various inbred strains of mice. IV. Comparative observation on virulence of four strains of *M. avium*. *Jap. J. Lepr. 50*:173–184, 1981 (in Japanese).

Kawaguchi, Y., M. Matsuoka, K. Sushida, and M. Tanemura. Susceptibility to *Mycobacterium avium* of various inbred strains of mice. V. Host resistance to *M. avium* of hybrids of C3H origin. *Jap. J. Lepr. 51*:1–8, 1982 (in Japanese).

Kawaguchi, Y., M. Matsuoka, K. Kawatsu, K. Sushida, and M. Tanemura. Susceptibility to *Mycobacterium avium* of various inbred strains of mice. VI. On development of the lesions in nude mice. *Jap. J. Lepr. 51*:9–16, 1982 (in Japanese).

Kawaguchi, Y., M. Matsuoka, and K. Kawatsu. Susceptibility to *M. lepraemurium* of CBA, DBA and C3H mice. *Jap. J. Lepr. 51*:57–64, 1982 (in Japanese).

Kawaguchi, Y. Attenuation of virulence of *M. lepraemurium* after serial mouse passages during long years. I. Development of murine leprosy lesions in mice with subcutaneous infection. *Jap. J. Lepr. 51*:117–128, 1982 (in Japanese).

Kawaguchi, Y. Attenuation of virulence of *M. lepraemurium* after serial mouse passages during long years. II. With special reference to classification of experimental mouse leprosy. *Jap. J. Lepr. 51*:129–134, 1982 (in Japanese)

Kawaguchi, Y. Attenuation of virulence of *M. lepraemurium* after serial mouse passages during long years. III. Survival time of inbred strains of mice after intraperitoneal infection with *M. lepraemurium*. *Jap. J. Lepr. 52*:147–155, 1983 (in Japanese)

Kawaguchi, Y. Attenuation of virulence of *M. lepraemurium* after serial mouse passages during long years. IV. Susceptibility to *M. lepraemurium*, strains Hawaiian-M and Hawaiian-B, of C57BL/6-nu/nu, C3H/He-nu/nu and BALB/c-nu/nu mice. *Jap. J. Lepr. 52*:187–194, 1983 (in Japanese)

Kawaguchi, Y. Attenuation of virulence of *M. lepraemurium* after serial mouse passages during long years. V. Pathogenicity of cultivated *M. lepraemurium*, strain Hawaiian-Ogawa. *Jap. J. Lepr. 53*:1–7, 1984 (in Japanese).

Kawaguchi, Y. Attenuation of virulence of *M. lepraemurium* after serial mouse passages during long years. VI. Virulence comparison test between Hawaiian-M and Hawaiian-B strains, using spread subcutaneous tissue preparations of mice. *Jap. J Lepr. 53*:47–53, 1984 (in Japanese)

Izumi, S., K. Sugiyama, Y. Matsumoto, and T. Nagai. Numerical changes in T cell subset ($T\gamma$ and $T\mu$) in leprosy patients. *Microbiol. Immunol. 24*:733–740, 1980

Izumi, S., K. Sugiyama, Y. Matsumoto, and S. Ohkawa. Analysis of the immunogenetic background of Japanese leprosy patients by the HLA system. *Vox Sang. 42*:243–247, 1982.

Izumi, S. and K. Sugiyama. Leprosy as a model of antigen specific immune disorders—A review. *Immunological Diseases (Japan) 3*:512–520, 1982.

Izumi, S., K. Sugiyama, Y. Matsumoto, K. Ohnishi, and T. Nagai. Studies on immunogenetic background in leprosy patients by analysis of HLA system. Progress in Immunology of Leprosy. (Talwar, G.O., J.L. Turk, and R.J.W. Rees, eds.). *Arnold-Heinemann Publishers, New Delhi*. 225–231, 1983

Izumi, S., S. Okada, and K. Sugiyama. Immunological changes in lepromatous leprosy by leucocyte transfusion. Progress in Immunology of Leprosy, (Talwar, G.P., J.L. Turk, and R.J.W. Rees, eds.). *Arnold-Heinemann Publishers, New Delhi*. 270–276, 1983.

Kohsaka, K., K. Yoneda, Y. Arimochi, M. Makino, T. Mori, and T. Ito. Nude Mouse as a Model for Chemotherapy of Leprosy. Proceedings of the Third International Workshop on Nude Mouse. 1982 Gustav Fisher New York, Inc., 59–65, 1982

Kohsaka, K., K. Yoneda, Y. Miyata, T. Ito, and S. Tanabe. Susceptibility of rhino mouse to *Mycobacterium leprae*. *Jap. J. Lepr. 52*:195–199, 1983 (in Japanese)

Kohsaka, K., K. Yoneda, T. Ito, and S. Tanabe. An attempt to inoculate *Mycobacterium leprae* into "Rhino" mice. *Expl Cell Biol. 52*:150–153, 1984

Koya, G., I. Arakawa, and N. Narita. Histopathological findings of serial preparation including the total length of nerve extrimitas thracica in leprosy. *Jap. J. Lepr. 49*:1–9, 1980 (in Japanese).

Koya, G. and N. Narita. Changes of amyloid-invaded intramucosal and muscularis mucosa nerves in the colon of leprosy—Observation with Golgi staining. *Jap. J. Lepr. 50*:39–45, 1981 (in Japanese)

Koya, G., N. Narita, and M. Akimoto. Mucous membrane-innervated nerve alteration caused by amyloid-deposited lammina muscularis mucosa in large intestine of lepromatous leprosy. *IGAKU NO AYUMI 117*:130–131, 1981 (in Japanese)

Koya, G. and M. Kudo. What a secondary degeneration is in the spinal posterior column. *IGAKU NO AYUMI 124*:14–15, 1983 (in Japanese)

Koya, G., N. Sasaki, and M. Kudo. Formation of neuropathological state in senile dementia of leprosy—An autopsy case of aged patient in Tn type. *Jap. J. Lepr. 52*:17–28, 1983 (in Japanese)

Kubo, E., Y. Fukunishi, M. Imaizumi, T. Matsumoto, S. Okada, T. Nagai, and M. Miyauchi. Determination of DDS level in blood. *Jap. J. Lepr. 52*:29–34, 1983 (in Japanese)

Kusaka, T., K. Kohsaka, Y. Fukunishi, and N. Akimori. Isolation and identification of mycolic acids in *Mycobacterium leprae* and *Mycobacterium lepraemurium*. *Int. J. Lepr. 49*:406–416, 1981.

Kusaka, T. and S. Izumi. Gaschromatography of constitutive fatty acids in *Mycobacterium leprae*. *Microbiol. Immunol. 27*:409–414, 1983

Matsuo, Y. and H. Tatsukawa. *In vitro* growth of *Mycobacterium lepraemurium* under the influence of macrophage cultures. *Microbiology and Immunol.*, *25*:801–805, 1981.

Matsuo, Y. and H. Tatsukawa. Attempts at cultivation of *Mycobacterium leprae* in cell culture under regulation of redox potential at environment. *Hiroshima J. Med. Sci.*, *31*:141–143, 1982.

Matsuo, Y. Use of cycloheximide for cultivating *Mycobacterium lepraemurium* in cell culture. *Int. J. Lepr. 51*:72–76, 1983

Matsuo, Y. Further studies of the use of cycloheximide for cultivating of *Mycobacterium lepraemurium* in cell culture. *Microbiology and Immunology*, *28*:1093–1098, 1984

Matsuoka, M. and Y. Kawaguchi. Observation on the host reaction to the cultivated *M. lepraemurium* in the spread subcutaneous tissue preparations of mice. *Jap. J. Lepr. 51*:96–106, 1982

Mori, T. and Mar-Mar-Nyein. Study of a growth factor for *Mycobacterium lepraemurium* grown in the Ogawa yolk medium. II. Egg yolk protein fraction and effect of reducing reagents. *Jap. J. Lepr. 50*:105–115, 1981.

Mori, T., Y. Miyata, K. Yoneda, and T. Ito. Collection method for *Mycobacterium leprae* from infected armadillo liver. *Int. J. Lepr. 52*:41–43, 1984.

Nakamura, K. and Y. Yogi. The nude mouse as an experimental lepromatous leprosy model: the enhancing effect of thymus cells in infected nude mice. *In*: Leprosy. (Latapi. F. et al eds.), Excerpta Medica, Amsterdam-Oxford-Princeton, 44–50, 1980.

Nakamura, M. and A. Kawabata. Relationship between preservation temperature and growth ability of *M. lepraemurium*. *Jap. J. Lepr. 49*:153–155, 1980.

Nakamura, M. and A. Kawabata. Deterioration of the growth promoting potentiality of cell-free liquid medium for *M. lepraemurium* during the incubation at 30°C. *Jap. J. Lepr. 49*:156–159, 1980.

Nakamura, M. and H. Ichimaru. Susceptibility test of *Mycobacterium lepraemurium* against Mycobacterio-phages. *Jap. J. Lepr. 49*:168–171, 1980.

Nakamura, M. Flask culture of *Mycobacterium lepraemurium*. *Jap. J. Lepr. 49*:172–175, 1980.

Nakamura, M. Enhanced growth of *Mycobacterium lepraemurium* in cell-free liquid medium with DL-aspartic acid. *The Kurume Medical Journal. 28*:315–318, 1981.

Nakamura, M. Growth stimulation of *Mycobacterium lepraemurium* in cell-free liquid medium by vitamin K_3 and B_{12}. *Jap. J. Lepr. 50*:135–138, 1981.

Nakamura, M. Effect of Pantoyl lactone on the growth of *Mycobacterium lepraemurium* in cell-free liquid medium. *Jap. J. Lepr. 50*:139–143, 1981.

Nakamura, M. Stimulating effects of lecithin and cholesterol on the growth of *Mycobacterium lepraemurium* in cell-free liquid medium. *Jap. J. Lepr. 51*:35–39, 1982.

Nakamura, M. Growth stimulation of *Mycobacterium lepraemurium* by liposome in cell-free liquid medium. *Jap. J. Lepr. 51*:40–43, 1982.

Nakamura, M. Stimulation of growth of *Mycobacterium lepraemurium* in cell-free liquid medium by DL-aspartic acid. *Intern. J. Lepr. 50*:193–199, 1982.

Nakamura, M. Stimulation effect of dextran on *in vitro* growth of *Mycobacterium lepraemurium*. *Jap. J. Lepr. 51*:81–84, 1982.

Nakamura, M. Effect of vitamins on the growth of *Mycobacterium lepraemurium* in cell-free liquid medium. *Jap. J. Lepr. 51*:173–178, 1982.

Nakamura, M. Effect of amino acids on the growth of *Mycobacterium lepraemurium* in cell-free liquid medium. *Jap. J. Lepr. 51*:179–182, 1982.

Nakamura, M.: Growth inhibition of *Mycobacterium lepraemurium* in cell-free medium by adding the suspension of *M. leprae*. *Jap. J. Lepr. 52*:111–116, 1983.

Nakamura, M. and T. Takeda. An attempt to modify the Hanks bacillary counting method: Hanks See-sand method. *Jap. J. Lepr. 52*:142–146, 1983.

Nakamura, M., Y. Matsuo, T. Mori, S. Okada, T. Nakayama, H. Saito, T. Ito, Y. Koseki, T. Ogawa, S. Ishihara, K. Kohsaka, K. Nakamura, and M. Ishidate. Failure to validate the growth of *Mycobacterium leprae* on M-Y 14b agar medium. *Int. J. Lepr. 50*:480–487, 1982.

Nakao, Y., H. Matsumoto, T. Miyazaki, H. Nishitani, K. Takatsuki, R. Kasukawa, S. Nakayama, S. Izumi, T. Fujita, and K. Tsuji. IgG heavy chain allotypes (Gm) in autoimmune diseases. *Clin. Exp. Immunol. 42*:20–26, 1980.

Nomaguchi, H., Mar-Mar-Nyein, K. Kohsaka, K. Yoneda, and T. Mori. The growth and drug sensitivity of *M. lepraemurium* by tissue culture applying monolayer and agar suspension technique. *Int. J. Lepr. 48*:277–284, 1980.

Nomaguchi, H., H. Wada, E. Wada, T. Mori, Y. Miyata, K. Kohsaka, and T. Ito. Antibody production of mice infected with *M. leprae* or *M. lepraemurium*. Proceedings of the Workshop on Serological Tests for Detecting Subclinical Infection in Leprosy. Sasakawa Memorial Health Foundation, Tokyo, 45–48, 1983.

Ozawa, T. On the infection and immunity of leprosy. *MINOPHAGEN Medical Review 27*:200–215, 1982 (in Japanese).

Saito, N. and Y. Hirooka. Evidence for generation of suppressor T cells in *Mycobacterium lepraemurium*-infected CBA/J mice. A mouse strain highly susceptible to the infection. *Microbiol. Immunol. 27*:75–85, 1983.

Saito, N. and T. Natori. Low responsiveness to MLM bacilli associated with an impaired macrophage function in CBA/J mice. *Clin. Immuno. Genetics*. in press, 1985.

Satoh, M., S. Izaki, S. Kon, and M. Izaki. Fibrin and collagen deposition and fibroblasts proliferation in granuloma of murine leprosy. Comparison of two mouse strains with different immune reactions. *Experientia 39*:899–902, 1983.

Samuel, N.M., J. Loudon, and K. Kohsaka. Growth of *Mycobacterium leprae* in nude mice. *Leprosy in India. 55*:648–653, 1983.

Suzuki, H., M. Narita, K. Onishi, T. Suzuki, Y. Sakamoto, and N. Saito. Studies on circulating immune complexes and anti-*M. leprae* antibody in patients with leprosy. *Jap. J. Lepr. 53*:15–22, 1984 (in Japanese).

Suzuki, H., M. Narita, K. Ohnishi, T. Suzuki, and N. Saito. Serum lysozyme and circulating immune complexes in patients with leprosy. *Jap. J. Lepr. 53*:23–27, 1984 (in Japanese).

Tanemura, M., K. Sushida, Y. Kawaguchi, and M. Matsuoka. Susceptibility to *Mycobacterium avium* of various inbred strains of mice. III. On the cases intraperitoneally infected with small dose. *Jap. J. Lepr. 50*:169–172, 1981 (in Japanese).

Tsukiyama, F., M. Katoh, and Y. Matsuo. Modification of the fluorescent staining method for mycobacterial cells. *Hiroshima J. Med. Sci., 33*:293–295, 1984.

Yamada, Y., E. Matsuo, K. Maeda, S. Tomizuka, and M. Mitani. Anti-human kidney β-glucuronidase antibody preparation and the enzyme extraction. *J. Kyorin Med. Soc. 11*:133–140, 1980 (in Japanese).

Yoneda, K. Studies on chemotherapy of leprosy. Screening of the drug against experimental leprosy in nude mice. Osaka Daigaku Igaku Zassi (*Medical Journal of Osaka University, Japanese Edition*), *33*:63–74, 1964.

U.S. Leprosy Panel

BROAD GOALS

Leprosy, or Hansen's disease, remains a disease of major importance in the world today. It remains one of the leading causes of disability and blindness in tropical countries. The worldwide prevalence of leprosy is estimated to be between 10 and 15 million cases with less than half the estimated cases receiving treatment. The disease is particularly common in Asia, India, sub-Saharan Africa, and central South America. In the United States, some 1,300 patients are known to be under treatment out of an estimated 3,000–4,000 patients. Approximately 250 new cases are diagnosed annually, most being immigrants from Latin America and Southeast Asia.

Major scientific problems confronting leprosy workers include a) the inability to cultivate the causative organism, *Mycobacterium leprae*; b) an uncertain proportion of strains of *M. leprae* which have developed resistance to one or more of the first line anti-leprosy drugs; c) the non-availability of an effective vaccine making case finding and case holding under chemotherapy the mainstay of leprosy control; d) lack of knowledge of the fundamental immunologic basis for susceptibility to leprosy; e) the persisting social stigma of the disease; f) the characteristic peripheral neuropathy of the disease with secondary crippling; and g) the fact that leprosy occurs predominantly in Third World Countries lacking the necessary resources to focus on what is already known about leprosy control. Many of these problems relate to social, economic, and public health fields. In scientific research, the developed world, including the United States and Japan, are actively engaged in research aimed at providing improved methods for leprosy control.

The major goals of the U.S.-Japan Leprosy Panels are:

1. Cultivation or growth of *M. leprae in vitro*;
2. Animal transmission and experimental systems for growth of *M. leprae*;
3. Evaluation of chemotherapeutic drugs against leprosy;
4. Development of chemoprophylaxis and minimal effective blood and tissue levels of drugs;
5. Vaccine development; and
6. Elucidation of antigens similar to lepromin and development of the mechanism of lepromin reactions.

Recently, the leprosy field has attracted a number of talented young investigators. This has been largely possible through the development of the armadillo animal model of lepromatous leprosy which has provided, for the first time, sufficient numbers of *M. leprae* to allow sophisticated research. Additionally, leprosy is being increasingly recognized as a model of infectious disease immunology. With more and more widespread use of the mouse footpad infection with *M. leprae*, tools have become available for monitoring drug resistance internationally.

SUMMARY OF THE THIRD FIVE YEARS

From 1975 to 1980, tremendous progress was made in leprosy research under the U.S.-Japan Leprosy Program and other international agencies. Large numbers of *M. leprae* from infected armadillos provided supplies of bacilli for a number of biochemical and immunochemical studies. Athymic nude rats and athymic nude mice were developed as models of lepromatous leprosy and utilized for a variety of chemotherapeutic tests. *Mycobacterium lepraemurium* was successfully cultivated *in vitro* and conditions optimized for growth.

In the field of chemotherapy, there was considerable interest in developing an effective therapeutic regimen using drug combinations instead of monotherapy. Rifampin was shown to be rapidly bactericidal for *M. leprae*. In animal systems, there was strong evidence that rifampin was synergistic with a number of other first line anti-leprosy drugs. Analytical methods were refined for assaying measures of dapsone in clinical materials. The possibility of carcinogenicity of dapsone was thoroughly considered; it was concluded that this represented an insignificant human risk when compared with the almost indispensable benefit of the drug for leprosy control.

There was considerable progress in immunologic studies aimed at the development of an effective vaccine against leprosy and in attempting to understand the basic immunologic deficit responsible for development of overt disease. Heat-killed *M. leprae* were shown to protect animals against subsequent challenge with viable *M. leprae* in mice and armadillos. Purification methods were developed for the bacilli from armadillo liver. Studies were initiated in developing a serologic test to identify individuals infected with *M. leprae* prior to overt signs or symptoms of the disease. There was considerable interest in studies of the basic immunologic defect in lepromatous leprosy; a hypothesis was proposed that suppressor T cells could be responsible for this unresponsiveness. In *M. lepraemurium* infections in rodents, it was shown that there are two populations of cells which function as suppressors, a T cell and an adherent cell, probably a macrophage. One group found evidence that in human lepromatous disease there were *M. leprae*-specific suppressor T cells and the hypothesis was suggested that these might be involved in the pathogenesis.

PROGRESS IN PRIORITY AREAS

The Microbiology of *M. Leprae*

Cultivation has remained a problem. A number of mycobacteria have been successfully cultivated from leprous tissues but a majority of leprosy workers believe that these are not truly *M. leprae*. Three types of organisms have been cultivated with some regularity. The first of these are mycobacteria which seem to be of the *M. avium-intracellulare-scrofulaceum* complex. The second are the so-called leprosy derived corynebacteria, and more recently, a third group are the so-called "difficult to grow mycobacteria obtained from *M. leprae*-infected armadillo tissues. At least two groups have undertaken cloning of *M. leprae* DNA into *E. coli* using recombinant DNA technology. There has been expression of cloned *M. leprae* DNA in *E. coli* in both laboratories within the last year. This raises the possibility and probability that relevant antigens of *M. leprae*, relevant enzymes important for drug development, and a host of other characteristics of *M. leprae* can be reproduced in the laboratory, even if the organism itself cannot be cultivated.

There has been enormous progress in the biochemistry of *M. leprae* over the last 5 years, mainly by British workers. Pathways normally associated with glycolosis, the tricarboxylic acid cycle, the hexose monophosphate pathway and glycerol catabolism have been identified. A major biochemical defect which prevents *in vitro* cultivation has not been identified. Lipid analysis of *M. leprae* has made remarkable progress in the last 5 years. The most significant finding is identification of a phenolic glycolipid on the surface of *M. leprae* and in tissues surrounding *M. leprae*. The terminal trisaccharide on this phenolic glycolipid is apparently unique to *M. leprae*. The phthiocerol core of this antigen is common to only a few species of mycobacteria including *M. kansasii*. The antigen has shown considerable promise in serologic assays and is now routinely utilized in a number of seroepidemiologic studies. There has been speculation that live *M. leprae in vivo* multiply with a generation time of approximately 26 hours instead of the traditional 2 weeks. More recently, it was shown that *M. leprae* is capable of generating increased amounts of ATP within a matter of minutes under certain culture conditions *in vitro*. A fluorescent method has been proposed for determining viability of *M. leprae*.

Experimental Infections in Leprosy

Armadillos continue to provide the source of *M. leprae* for investigators worldwide. Armadillos in Louisiana and Texas have been shown to have natural infections with *M. leprae*. The prevalence of these infections is approximately 4% in Texas. The source of these infections is unknown, but there is serologic evidence suggesting that the infection has been present in Louisiana armadillos as early as 1961. (The first experimental inoculations of armadillos with *M. leprae* occurred in 1968.) Spontaneous leprosy has been reported in a mangabey monkey and has been successfully transmitted to a substantial number of mangabey monkeys over the past several years. Some successful transmissions have occurred with African green monkeys but limited success has been observed with rhesus monkeys and no success with squirrel monkeys. This primate model now offers considerable promise in the study of peripheral nerve damage, immunologic studies and a number of other aspects of human disease. Nude mice have been used increasingly in leprosy research in both the U.S. and Japan. This model has now been exploited in a number of ways, including studies such as transmission of leprosy, vaccine development, and drug efficacy.

Chemotherapy

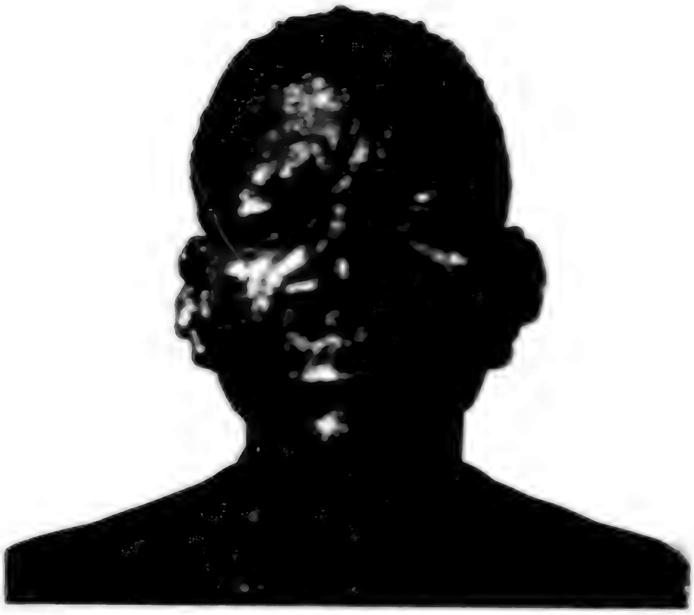
There have been a number of developments in chemotherapy of leprosy. Drugs routinely available for antibacterial chemotherapy include dapsone, rifampin, clofazimine, and the thioamides—prothionamide and ethionamide. Anti-reaction drugs are now available such as thalidomide for erythema nodosum leprosum, clofazimine for erythema nodosum leprosum and reversal reactions. Corticosteroids assist in management of erythema nodosum leprosum and reversal reactions. Within the last 5 years, triple and quadruple drugs are recommended for chemotherapeutic regimens. More conservative approaches are undertaken in other international centers.

Secondary sulfone resistance was detected in the 1960's, and in the 1970's primary sulfone resistance, secondary ethionamide resistance and secondary resistance to rifampin. A single case of clofazimine resistance was described in 1983. Alarm has been expressed over the apparent increase of primary dapsone resistance and the apparent increase in secondary resistance to dapsone. A number of international studies of compliance in leprosy chemotherapy show poor compliance, particularly after the first year of chemotherapy.

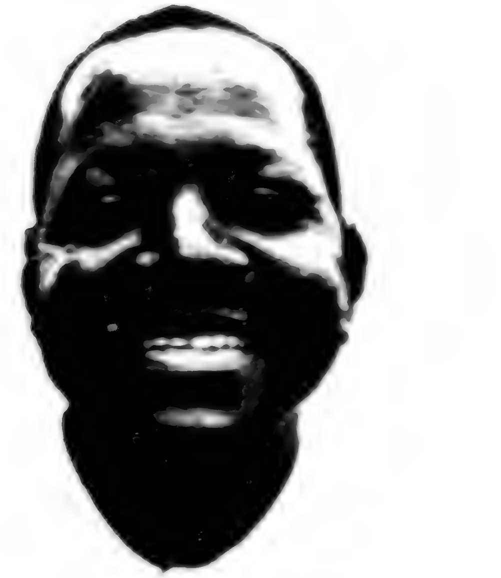
A number of significant findings in the treatment of leprosy were reported during the past several years. Efforts were made to improve existing individual anti-leprosy drugs: a) attempts to develop analogs of thalidomide, not teratogenic and effective in erythema nodosum leprosum; b) attempts to develop analogs of clofazimine which lacked pigmenting and G.I. side effects and which are otherwise safe and effective against *M. leprae* and in reactions have been unsuccessful; c) the thioamides are optimal in their antibacterial activity and safety. They act via the same mechanism as thiambutazine and thiacetazone; d) rifampin analogs possess anti-leprosy activity. One analog, ansamycin, is active against rifampin-resistant *M. leprae* in mouse footpad infections; e) dapsone is optimal for activity against *M. leprae* based on structure activity relationship studies. The extraordinary sensitivity of *M. leprae* to dapsone is due to the high affinity of its dihydropteroate synthetase for dapsone; f) drug combinations which include daily rifampin plus daily thioamide result in additive hepatotoxicity with up to 15% of patients developing drug-induced hepatitis. Thus, combination chemotherapy regimens cannot employ rifampin plus a thioamide on a daily basis; and g) continuous rifampin administration rather than a variety of intermittent regimens, is necessary to eliminate persisting *M. leprae* in infected neonatally thymectomized Lewis rats.

Immunology

In the last 5 years, interesting studies dealt with the transmission of leprosy, suppressor mechanisms in illness, and reactional states. Lepromin skin testing of armadillos resulted in histopathologic findings of tuberculoid, borderline, and lepromatous patterns. Interestingly, all three patterns occur in animals which are resistant to infection with *M. leprae* as evidenced by long-term survival despite effective i.v. inoculation with high doses of viable bacilli. This raises the possibility



Patient with lepromatous leprosy.



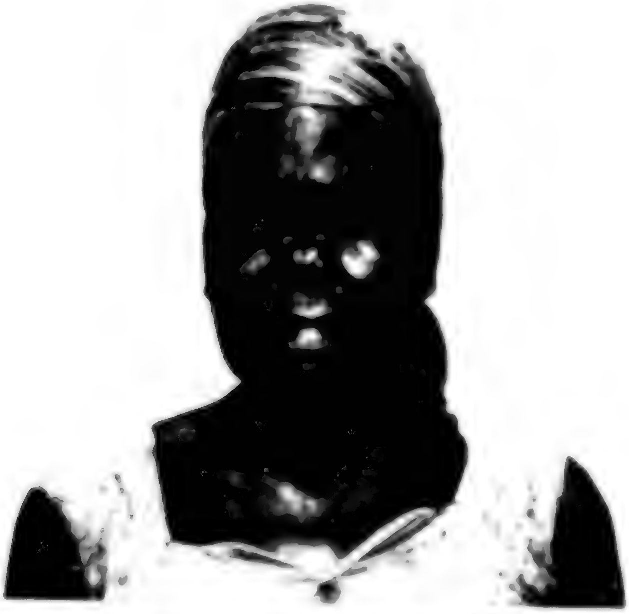
After 2 years' treatment with dapsone.

that animals with innate resistance to leprosy may not need to develop a cell-mediated immune response to *M. leprae* in order to express that resistance.

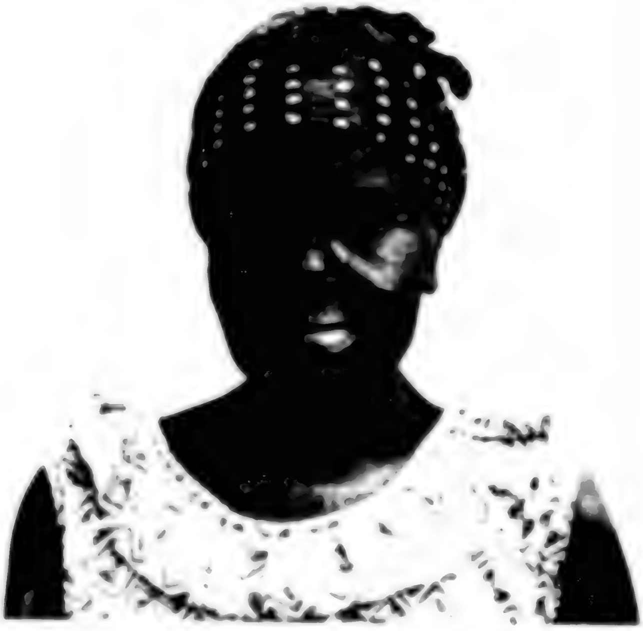
There is considerable promise that persons infected with *M. leprae* can be identified prior to the onset of overt illness. This was suggested in field seroepidemiologic studies utilizing an ELISA technique to detect IgM class antibody to the phenolic glycolipid antigen of *M. leprae*. Among 112 household contacts of leprosy patients, 18 were consistently seropositive over a 2½ year period. Two of these 112 contacts developed tuberculoid leprosy during the period of observation and each patient was within the sub-group of 18 contacts who showed consistently elevated antibody titers. A dot ELISA was developed to detect *M. leprae*-specific phenolic glycolipid in clinical specimens.

In studies from Ethiopia, there was strong evidence for placental transmission of leprosy from active lepromatous mothers to their babies. In cord sera of babies born to lepromatous mothers, 30% contained IgA class anti *M. leprae* antibodies and 50% contained IgM class anti *M. leprae* antibodies. No cord sera from babies of tuberculoid mothers or non-leprosy control mothers had detectable antibodies to *M. leprae*.

There is a strong interest in searching for suppressor mechanisms in leprosy. Data from Norway suggests that cell-mediated immunologic unresponsiveness in lepromatous leprosy is commonly due to a deficiency in the production of



Patient with leproma in nose, causing disfiguration.



After nose was surgically reconstructed using bone and cartilage from ribs.

interleukin-2 in related factors, and is not due to a lack of *M. leprae*-reactive T cells. Other groups find this pattern only in borderline lepromatous patients and are unable to demonstrate any *M. leprae*-reactive T cells in polar lepromatous disease. In several laboratories, suppressor cells were present in most normal donors who are lepromin positive or in most tuberculoid leprosy patients. They were not demonstrated in lepromatous leprosy patients. Many conclude that suppressor mechanisms appear to reflect a normal mechanism which is diminished rather than increased in lepromatous patients. Elegant studies revealed helper and suppressor T cell distributions in leprous skin lesions (see 17th Joint Leprosy Conference, Sendai, Japan). In leprous skin lesions interleukin-2 producing cells are ten-fold more abundant in BT lesions than in lepromatous lesions; the number of anti-Tac positive are similar in both. These findings suggest that antigen has been presented to helper T cells in lepromatous leprosy but that IL-2 producing T cells are reduced. Presumably, the basic deficit in specific cell-mediated immunity in lepromatous leprosy is due to reduced IL-2 production (see 19th Joint Conference, Tokyo, Japan).

In studies of the immunopathogenesis of reactional states in leprosy, high antibody activity was significantly correlated with active skin lesions, new skin lesions, and neuritis in patients with borderline tuberculoid disease. These findings confirm that there is not always an inverse relationship between cell-mediated immunity and humoral immune responses to *M. leprae*. Markedly depressed natural killer (NK) cell activities were described in patients with erythema nodosum leprosum. There is evidence that the patients' monocytes were responsible for this depression in NK cell activity. C3d was shown to be markedly increased in erythema nodosum leprosum as well as the concentration of factor B breakdown product (Ba) in relation to factor B. Thus, erythema nodosum leprosum may be associated with complement activation and the alternative pathway may be the mechanism for complement activation in erythema nodosum leprosum. The number of suppressor T cells in the circulation decreases prior to the onset of erythema nodosum leprosum; they reach abnormally high numbers in lepromatous leprosy patients with clinical recovery from erythema nodosum leprosum. Soluble extracts of ultrasonically disrupted mycobacteria, including *M. leprae*, cause strong T cell-dependent polyclonal B cell activation in peripheral blood mononuclear cell preparations from normal humans. Hence, erythema nodosum leprosum is characterized by a drop in functional activity of suppressor T cells which may then allow expression of the underlying type III hypersensitivity mechanisms. These may then involve the alternative pathway of complement activation as well as the classical pathway. Elevated levels of phenotypically suppressor T cells in the circulation in lepromatous leprosy may well represent a homeostatic mechanism which acts on B cell proliferation to control ENL.

FUTURE GOALS

Microbiology of *M. leprae*

The *in vitro* cultivation of *Mycobacterium leprae* remains a major goal in leprosy research. In recent years, the development of radiolabeling, ATP assay, and fluorescent staining as measures of *in vitro* viability of *M. leprae* have provided tools which can be exploited in a systematic, but conventional way to approach cultivation. In the interim, the expression of *M. leprae* genes in *E. coli* by recombinant DNA technology may allow relevant antigenic and biochemical analyses. Armadillos will provide sources of leprous bacilli in the absence of cultivation or recombinant DNA expression.

Experimental Infections of Leprosy

Exploitation of the primate model of leprosy should provide relevant information regarding human immunology, the pathogenesis of nerve damage in human leprosy, and routes of transmission of human disease. The nude mouse will be further exploited in studies of the transmission of leprosy, the pathogenesis of erythema nodosum leprosum, chemotherapeutic studies, and macrophage kinetics in lepromatous granuloma.

Chemotherapy

The search for new drugs with anti-*M. leprae* activity will continue as opportunities present. Major responsibility has been undertaken by the THELEP component of the World Health Organization and results are not extraordinarily promising. Longitudinal monitoring of primary dapsone resistance continues in the United States. Chemotherapeutic trials in experimental animal models will continue. A multi-center clinical trial of ansamycin is planned. Interest continues in the development of dihydrofolate reductase inhibitors, B-lactamase inhibitors in combination with B-lactam antibiotics, the newer cephalosporins, and newer anthracene derivatives as anti-leprosy chemotherapeutic agents.

Immunology

The immunochemistry of *M. leprae* will continue to be elucidated. Synthetic antigens based on the terminal disaccharide of the phenolic glycolipid antigen of *M. leprae* were developed and will be utilized in serology and seroepidemiology. Other lipid antigens, protein antigens, and a variety of monoclonal antibodies are either already available or will soon become available for these purposes. T cell clones for study of cell-mediated immune mechanisms in leprosy are becoming available and should be exploited over the next few years. Further immunochemical analysis of clinical leprous lesions should yield significant new findings. A number of field seroepidemiologic studies should continue to bear fruit over the coming years. The IMMLEP component of the World Health Organization is poised to undertake clinical trials of a leprosy vaccine. Phase I trials of purified armadillo derived *M. leprae* have been completed in Norway and the United Kingdom and are now underway in the United States.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
GRANTS	
Barry R. Bloom Yeshiva University	Immunologic studies in leprosy
Patrick J. Brennan Colorado State University	*Mycobacterium leprae* glycolipid antigens
Thomas M. Buchanan University of Washington	Serodiagnosis of subclinical leprosy
Frank M. Collins Trudeau Institute, Inc.	A mouse infection model of lepromatous leprosy
Arvind M. Dhople Florida Institute of Technology	Application of ATP assays to patient care in leprosy
Howard A. Fieldsteel SRI International	Cultivation of *M. leprae* in tissue culture
Thomas P. Gillis National Hansen's Disease Center	Antigenic analysis of *M. leprae* with monoclonal antibody
Bobby J. Gormus Tulane University	Characterization of experimental leprosy
John H. Hanks Johns Hopkins University	Energetics and cultivation of *M. leprae*
Robert C. Hastings National Hansen's Disease Center	Nude mice in leprosy research
Timothy J. Holzer University of Illinois at Chicago	Antimycobacterial activity of phagocytic cells
James L. Krahenbuhl SRI International	Immunology and immunotherapy of experimental leprosy
James L. Krahenbuhl National Hansen's Disease Center	Immunology and immunotherapy of leprosy
Maurice J. Lefford Wayne State University	Immunological problems of leprosy
Robert L. Modlin University of Southern California	Immunoregulation in leprosy granulomas
Ramchandra G. Navalkar Meharry Medical College	Immunological studies on leprosy
Kenrad E. Nelson University of Illinois Medical Center	Immunobiology and epidemiology of leprosy
Parsotam J. Patel Meharry Medical College	Host-parasite interactions in murine leprosy
John H. Peters SRI International	Studies of anti-leprosy drugs in animals and humans

Investigator and Institution	*Title*
Zulema R. Reggiardo University of California, Davis	Serological diagnosis of leprosy
Eleanor E. Storrs Florida Institute of Technology	Comparative susceptibility of armadillos to leprosy
CONTRACTS	
Patrick J. Brennan Colorado State University	The isolation, purification, and characterization of antigens from purified *Mycobacterium leprae* obtained from armadillo tissues
Thomas M. Buchanan University of Washington	The isolation, purification, and characterization of antigens from purified *Mycobacterium leprae* obtained from armadillo tissues
Robert C. Hastings National Hansen's Disease Center	Establishment and maintenance of an armadillo colony infected with *M. leprae*
Charles Shepard Centers for Disease Control	Pharmacology of anti-leprosy drugs
Eleanor E. Storrs Florida Institute of Technology	Establishment and maintenance of an armadillo colony infected with *M. leprae*

BIBLIOGRAPHY

Amezcua, M.A., A. Escobar-Gutierrez, E.E. Storrs, A.M. Dhople, and H.P. Burchfield. Wild Mexican armadillo with leprosy-like infection. *Int. J. Lep. 52*:254–255, 1984.

Athwal, R.S., S.S. Deo, and T. Imaeda. Deoxyribonucleic acid relatedness among *Mycobacterium leprae, Mycobacterium lepraemurium* and selected bacteria by dot blot hybridization and spectrophotometric deoxyribonucleic acid assays. *Int. J. Syst. Bacteriol. 34*:371–375, 1984.

Barksdale, L. Leprosy Vaccines. *Int. J. Lep. 51*:107–110, 1983.

Barksdale, L. "Leprosy" In *Corynebacterium, Mycobacterium and Nocardia*. Medical Microbiology, Samuel Baron, ed., Addison-Wesley, Publishers, 1985.

Barnhill, R.L., N.J. Doll, and R.C. Hastings. Studies on the anti-inflammatory properties of thalidomide: Effects on polymorphonuclear leukocytes and monocytes. *J. Am. Acad. Dermatol.* (in press).

Baskin, G.B., R.H. Wolf, B.J. Gormus, L.N. Martin, P.J. Gerone, W.M. Meyers, G.P. Walsh, and C.H. Binford. Naturally acquired and experimental leprosy in monkeys. *Lab. Invest. 50*:40A, 1984.

Baskin, G.B., R.H. Wolf, B.J. Gormus, L.N. Martin, G.P. Walsh, C.H. Binford, W.M. Meyers, and R. Malaty. Experimental leprosy in the mangabey *(Cercocebus atys)*: Necropsy findings. *Int. J. Lep. 53*, March issue, 1985, in press.

Binford, C.H. Leprosy. In *Anderson's Pathology*, Eighth edition. ed. John M. Kissane. St. Louis, MO. The C. V. Mosby Company, Two volumes. Volume *1*:322–334, 1985.

Binford, C.H., W.M. Meyers, and G.P. Walsh. Leprosy—State of the Art. *J.A.M.A. 247*:2283–2292, April 23-30, 1982.

Bloom, B.R., V. Mehra, C. Grosskinsky, and C. Brosnan. Immunology of Leprosy. *In* Progress in Immunology V. Academic Press, Japan, Inc. pp. 1279-1293, 1983.

Bloom, B.R. and V. Mehra. The pathogenesis of lepromatous leprosy. In Immunological Aspects of Leprosy, Tuberculosis and Leishmaniasis (D. P. Humber, ed.). International Congress Series 574. *Excerpta Medica*, pp. 128–137, 1981.

Bloom, B.R. and V. Mehra. Vaccine strategies for the erradication of leprosy. In *New Approaches to Vaccine Development*. Torrigiani and Asaad, ed. 1983, in press.

Bloom, B.R. and V. Mehra. Immunological Unresponsiveness in Leprosy. *Immunol. Rev. 80*:5–28, 1984.

Bloom, B.R. Rationales for vaccines against leprosy. *Int. J. Lep. 51*:505–509, 1982.

Bloom, B.R. and T. Godal. Selective Primary Health Care: Strategies for control of disease in the developing world. V. Leprosy. *Rev. Infect. Dis. 5*:765–779, 1983.

Brand, P.W., R.B. Beach, and D.E. Thompson. Relative Tension and Potential Excursion of Muscles in the Forearm and Hand. *J. Hand Surg. 6*(3):209–219, 1981.

Brand, P.W. The Foot in Diabetes Mellitus. In *Diabetes Mellitus: Diagnosis and Treatment*. Harold H. Rifkin and P. Raskin eds., Prentice-Hall Publishing and Communications Co., 1981.

Brand, P.W. Tendon Transfers in the Forearm. In *Hand Surgery*, 3rd ed.: J.E. Flynn, ed. Williams and Wilkins Co., Baltimore, MD. 1981.

Brand, P.W. The Hand in Leprosy. In *The Hand*: Tubiana, R., ed. W.B. Saunders Company, 1983.

Brand, P.W. The Insensitive Foot. In *Disorders of The Foot*: Jahss, M.H., ed. W.B. Saunders Company, Philadelphia, PA. 1266–1286, 1982.

Brand, P.W. The Mechanics of Dynamic Splinting. In *Current Concepts in Hand Surgery*: J.A. Boswick, ed. Lea and Febiger, 6–12, 1983.

Brand, P.W. Mechanics of Tendon Transfer. In *Current Concepts In Hand Surgery*, Boswick, J.A., ed., Lea and Febiger, 13–18, 1983.

Brand, P.W. Hand Rehabilitation—Management by Objectives In *Rehabilitation of The Hand*: Hunter, Schneider and Mackin, eds. C.V. Mosby Publishers, 1984.

Brand, P.W. Mechanics of Tendon Transfers. In *Rehabilitation of The Hand*: Hunter, Schneider and Mackin, eds. C.V. Mosby Publishers, 1984.

Brand, P.W. The forces of dynamic splinting: ten questions before applying a dynamic splint to the hand. In *Rehabilitation of the Hand*: Hunter, Schneider and Mackin, eds., C.V. Mosby Publishers, 1984.

Brennan, P.J. and W.W. Barrow. Evidence for species-specific lipid antigens in *Mycobacterium leprae. int. J. Lep. 48*:382–387, 1980.

Brennan, P.J. Leprosy Vaccine. *Science 216*:23, 1982. Letter.

Brennan, P.J. The phthiocerol-containing surface lipids of *Mycobacterium leprae. Int. J. Lep. 51*:387–396, 1983.

Brennan, P.J. *Mycobacterium leprae*—the outer lipoidal surface. *J. Bioscience*, in press.

Brown, S., M.A. Laneelle, J. Asselineau, and L. Barksdale. The Leprosy Derived Corynebacteria (LDC): A description of *Corynebacterium tuberculostericum*. *Annales de Microbiologie*. In press, 1985.

Brown, A., K.E. Nelson, S. Makonkawkeyoon, V. Vithayasi, D. Scollard, and W. Bullock: A study of the Immunological Effects of Cimetidine in Patients with Lepromatous Leprosy. *Amer. J. Trop. Med. and Hyg.* In press.

Buchanan, T.M., D.B. Young, R.A. Miller, S.R. Khanolkar. Serodiagnosis of infection with *Mycobacterium leprae*. *Int. J. Lep. 51*:524–530, 1983.

Buchanan, T.M. Prospects for Early Diagnosis, Immunotherapy and Vaccination for Leprosy. In *Microbiology*, American Society for Microbiology, Washington, DC, 1984.

Buchanan, T.M. Immunochemical and Diagnostic Uses of Monoclonal Antibodies in Leprosy and Gonorrhea. In *Hybridoma Technology In Agricultural Veterinary Research*, Stern and Gamble, eds., Rowman and Allenheld Publisher, 1984.

Bullock, W.E. Immunology of leprosy. In *Immunology of Human Infection, Part I, Chapter 15*, A. Nahmias and R. O'Reilly, eds., Plenum Publishing Corporation, NY, 1981.

Bullock, W.E. Summary of workshop on Infection-or-Adjuvant-Induced Alteration of the Immune Response. In *Immunoglobulin Genes and B Cell Differentiation*, J.R. Battisto and K.L. Knight, eds. Elsevier, North-Holland, NY, 1981.

Bullock, W.E. Leprosy, In *Cecil Textbook of Medicine*, Beeson, McDermott and Wyngarden, eds., 16th Edition, W.B. Saunders Company, Philadelphia, PA, 1982.

Bullock, W.E., S.R. Watson, K.E. Nelson, V. Schauf, S. Makonkawkeyoon and R.R. Jacobson. Aberrant Immunoregulatory Control of B. Lymphocyte Function in Lepromatous Leprosy. *Clin. Exp. Immunol.*, *49*:105–114, 1982.

Bullock, W.E. Rifampin in the Treatment of Leprosy. *Rev. Infect. Dis. 5*(Suppl. 3):606–613, 1983.

Bullock, W.E. The Lymphoid System in Infections with Mycobacteria. In *Advances in Host Defenses (Volume II), The Role of Lymphoid Cells in Host Defense Mechanisms*, J.I. Gallin and A.S. Fauci, eds., Raven Press, NY, 1983.

Bullock, W.E. Diagnosis and management of leprosy. In *Principles and Practice of Infectious Disease*. Second Edition. eds., G.L. Mandell, R.G. Douglas and J.E. Bennett. John Wiley and Sons, Inc., NY, 1984.

Bullock, W.E. Leprosy, In *Cecil Textbook of Medicine*, Beeson, McDermott and Wyngarden, eds., 17th Edition, W.B. Saunders Company, Philadelphia, PA, in press.

Bullock, W.E. Immunology of Mycobacterial Infections. In *Immunological Diseases*, Fourth Edition, ed., M. Samter, Little Brown and Co., Boston, MA, in press.

Chehl, S., J. Ruby, C.K. Job, and R.C. Hastings. The growth of *Mycobacterium leprae* in nude mice. *Lep. Rev. 54*:283–304, 1983.

Cho, S-N., T. Fujiwara, S.W. Hunter, T.H. Rea, R.H. Gelber, and P.J. Brennan. Use of an artificial antigen containing the 3,6-di-O-methyl-B-D glucopyranosyl epitope for the serodiagnosis of leprosy. *J. Infect. Dis. 150*:311–322, 1984.

Cho, S-N., D.L. Yanagihara, S.W. Hunter, R.H. Gelber, and P.J. Brennan. Serological specificity of phenolic glycolipid-I from *Mycobacterium leprae* and use in serodiagnosis of leprosy. *Infect. Immun. 41*:1077–1083, 1983.

Cho, S.-N., D.L. Yanagihara, S.W. Hunter, R.H. Gelber, and P.J. Brennan. Serological specificity of phenolic glycolipid-I from *Mycobacterium leprae* and use of serodiagnosis in leprosy. *Int. J. Lep. 51*:659–660, 1983.

Clark-Curtiss, J.E., W.R. Jocobs, M.A. Docherty, L.R. Ritchie, and R. Curtiss III. Molecular analysis of the DNA and construction of genomic libraries of *Mycobacterium leprae*. *J. Bacteriol.*, 1985. In press.

Collins, F.M., N.E. Morrison, A.M. Dhople, and S.R. Watson. Microscopic counts carried out on *Mycobacterium leprae* and *M. Tuberculosis* suspensions. A comparison of three staining procedures. *Int. J. Lep. 48*:402–407, 1980.

Collins, F.M., N.E. Morrison, and S.R. Watson. Fernandez and Mitsuda reactivity in guinea pigs sensitized with heat-killed *Mycobacterium leprae*: persistence and specificity of skin reactivity to soluble and particulate antigens. *Int. J. Lep. 51*:481–489, 1983.

Collins, F.M. and L.W. Poulter. Effector and escape mechanisms in tuberculosis and leprosy. In *Immunological Aspects of Leprosy, Tuberculosis and Leishmaniasis*. D.P. Humber, ed. Excerpta Medica, Amsterdam, pp. 97–113, 1981.

Dawson, P.J., M.J. Colston, and A.H. Fieldsteel. Infection of the congenitally athymic rat with *Mycobacterium leprae*. *Int. J. Lep. 51*:336–346, 1983.

Dhople, A.M. and J.H. Hanks. Pedigreed Stocks of *M. lepraemurium* for Cultivation and Metabolic Studies. *Can. J. Microbiol. 26*:1247–1252, 1980.

Dhople, A.M. and J.H. Hanks. Role of Sulfhydryls in the *In Vitro* Growth of *M. lepraemurium*. *Infect. Immun. 31*:352–357, 1981.

Dhople, A.M., Effect of Antibacterial Compounds on the *In Vitro* Growth of *M. leprae*. *Int. J. Lep. 49*:57–59, 1981.

Dhople, A.M. Effect of Antibacterial Compounds on the *In Vitro* Growth of *M. leprae*. *Microbios Letters. 14*:81–85, 1981.

Dhople, A.M. and E.E. Storrs, Effect of Purification Procedures on the ATP content of *M. leprae*. *Int. J. Lep. 50*:83–89, 1982.

Dhople, A.M. Effect of Freezing *M. leprae* in Tissues. *Leprosy in India*. *54*:461–470, 1982.

Dhople, A.M. Taxonomic Studies on *M. leprae*. *Leprosy in India*. *55*, 1983.

Dhople, A.M. Effect of Lysozyme on *M. leprae*. *Leprosy in India*. *55*, 1983.

Dhople, A.M. ATP Content of *M. leprae* from leprosy patients. *Int. J. Lep.* *52*:183–188, 1984.

Dhople, A.M., et al. Wild Mexican Armadillo with Leprosy-like infection. *Int. J. Lep.* *52*:254–255, 1984.

Dhople, A.M. and E.E. Storrs. Effect of purification procedures on the ATP content of *M. leprae*. *Int. J. Lep.* *50*:83–89, 1982.

Dhople, A.M., P.C. Howell, S.L. Williams, J.A. Zeigler, and E.E. Storrs. Serum-angiotensin-converting enzyme in leprosy. *Indian J. Lep.*, in press.

Dhople, A.M., K.J. Johnson, S.L. Williams, J.A. Zeigler, C.A. Cook, and E.E. Storrs. Nicotinamide adenine dinucleotide glycohydrolase in normal and leprous armadillos. In *Microbios Letters*, in press.

Dhople, A.M. and J.H. Hanks. *In vitro* growth of *Mycobacterium lepraemurium*: Transition from *in vivo* grown to *in vitro* adaptation. *The Kurune Medical Journal 27*:148–155, 1980.

Dhople, A.M. and J.H. Hanks. Effect of cholesterol, lecithin and nucleotides on the *in vitro* growth of *Mycobacterium lepraemurium*. *Japan J. Exp. Med.* *50*:469–471, 1980.

Dhople, A.M. and J.H. Hanks. Adenosine triphosphate content in *Mycobacterium leprae*. A brief communication. *Int. J. Lep.* *49*:57, 1981.

Douglas, J.T., S.O. Naka, and J.W. Lee. Development of an ELISA for detection of antibody and leprosy. *Int. J. Lep.* *52*:19–25, 1984.

Douglas, J.T. and R.M. Worth. Field evaluation of an ELISA to detect antibody in leprosy patients and their contacts. *Int. J. Lep.* *52*:26–33, 1984.

Fieldsteel, A.H. and L. Levy. Combined rifampin and dapsone chemotherapy of *Mycobacterium leprae* infection of the neonatally thymectomized Lewis rat. *Int. J. Lep.* *48*:267–276, 1980.

Fieldsteel, A.H., M.J. Colston, and N. Sato. Comparison of the neonatally thymectomized Lewis rat (NTLR) and the congenitally athymic nude rat with respect to infection with *Mycobacterium leprae*. *Int. J. Lep.* *48*:491–492, 1980.

Fieldsteel, A.H., N. Sato, and M.J. Colston. Relationship between T-cell populations in neonatally thymectomized Lewis rats and suceptibility to infection with *Mycobacterium leprae*. *Int. J. Lep.* *49*:317-323, 1981.

Fieldsteel, A.H. and M.J. Colston. The intact mouse as a host for the detection of small numbers of heat-killed *M. leprae*. *Int. J. Lep.* *49*:499, 1981.

Folse, D.S. and J.H. Smith. Leprosy in Wild Armadillos *(Dasypus novemcinctus)* of the Texas Gulf Coast: Anatomic pathology. *J. Ret. Soc.* *34*:341–357, 1983.

Frey, H., A. Gerson, and W.E. Bullock: Fatal dapsone syndrome in a patient treated for lepromatous leprosy. *Ann. Int. Med.* *94*:77, 1981.

Fujiwara, T., S.W. Hunter, S-N. Cho, G.O. Aspinall, and P.J. Brennan. Chemical synthesis and serology of disaccharides and trisaccharides of phenolic glycolipid antigens from the leprosy bacillus and properties of a disaccharide protein conjugate for serodiagnosis of leprosy. *Infect. Immun.* *43*:248–252, 1984.

Fukunishi, Y., W.M. Meyers, C.H. Binford, G.P. Walsh, F.B. Johnson, P.J. Gerone, R.H. Wolf, B.J. Gormus, and L.N. Martin. Electron microscopic study of leprosy in a mangabey monkey (Naturally acquired infection). *Int. J. Lep.* *51*:666–667, 1983.

Fukunishi, Y., W.M. Meyers, C.H. Binford, G.P. Walsh, F.B. Johnson, P.J. Gerone, R.H. Wolf, B.J. Gormus, and L.N. Martin. Electron microscopic study of leprosy in a mangabey monkey (Natural infection). *Int. J. Lep.* *52*:203–207, 1984.

Fukunishi, Y., W.M. Meyers, G.P. Walsh, F.B. Johnson, C.H. Binford, S. Okada, and M. Nishiura. Ultrastructural features of macrophages of armadillos infected with actively multiplying *Mycobacterium leprae*. *Int. J. Lep.* *52*:198–202, 1984.

Gad, S.M., E.J. Shannon, W.A. Krotoski, and R.C. Hastings. Thalidomide induces imbalances in T-lymphocyte subpopulations in the circulating blood of healthy males. *Lep. Rev.*, April 1985, in press.

Gaylord, H. and P.J. Brennan. Leprosy: antigens and immunity. In *Parasites, Antigens, Receptors and the Immune Response*. T.W. Pearson, ed. Marcel Dekker, Inc., New York, in press.

Geiseler, P., K. Nelson, and R. Crispen. Active tuberculosis in physicians. *JAMA*, 1984, in press.

Gelber, R.H. The activity of cycloserine and structurally related compounds against *M. leprae*-infected mice. *Int. J. Lep.*, in press.

Gelber, R.H. Leprosy. In *Dermatopharmacology*, Maibach and Gomez, Eds., C.V. Mosby Company, St. Louis, MO, 1985, in press.

Gelber, R.H. Primary dapsone-resistant leprosy in San Francisco. *Int. J. Lep.*, in press.

Gelber, R.H., P.R. Henika, and J.B. Gibson. The bactericidal activity of various aminoglycoside antibiotics against *Mycobacterium leprae* in mice. *Lep. Rev.*, in press.

Gillis, T.P., M. Abe, W.E. Bullock, O. Rojas-Espinosa, E. Garcia-Ortigoza, P. Draper, W. Kirchheimer and T.M. Buchanan. Comparison of 22 species of mycobacteria by immunodiffusion against and absorbed reference leprosy sera. *Int. J. Lep. 49*:287-293, 1981.

Gillis, T.P. and T.M. Buchanan. Production and partial characterization of monoclonal antibodies to *Mycobacterium leprae*. *Inf. Immun. 37*:172-178, 1982.

Gillis, T.P. and T.M. Buchanan. Monoclonal antibodies to *Mycobacterium leprae*: Production and partial characterization. *Infect. Immun. 37*:172-178, 1982.

Gormus, B.J., L.N. Martin, R.H. Wolf, G.B. Baskin, P.J. Gerone, W.M. Meyers, G.P. Walsh, H.L. Brown, C.H. Binford, C.J. Schlagel, and T.L. Hadfield. Immunologic effects of leprosy in mangabey monkeys *(Cerocebus atys)*. *Int. J. Lep. 52:* 1984, in press.

Graham, L. and R.G. Navalkar. Evaluation of *Mycobacterium leprae* immunogenicity via adoptive transfer studies. *Infect. Imm. 43*:79, 1984.

Graham, L. and R.G. Navalkar. Immune response in BALB/c mice following immunization with heat-killed *Mycobacterium lepraemurium*. *Zbl. Bakt. Infekt. Hyg. 257*:121, 1984.

Harris, E.B. and K. Prabhakaran. Quantitative study of the separation of *Mycobacterium leprae* from armadillo tissue. *Lep. Rev. 51*:359-362, 1980.

Hastings, R.D. Kellersberger Memorial Lecture 1979: Immunosuppressive/Anti-inflammatory thalidomide analogues. *Ethiop. Med. J. Med. 18*:65-71, 1980.

Hastings, R.C. The 1980 Journal—A perspective in leprosy. *Int. J. Lep. 49*:73-82, 1981.

Hastings, R.C., S.C. Chehl, G. Black, M.J. Morales, E.J. Shannon, and W.F Kirchheimer. Growth of *M. leprae* in nude (athymic) mice. In *Immunological Aspects of Leprosy, Tuberculosis and Leishmaniasis*. David P. Humber, ed., *Excerpta Medica*, Amsterdam-Oxford-Princeton, pp. 166-176, 1981.

Hastings, R.C. The 1981 Journal. A Continuing Perspective. *Int. J. Lep. 50*:102-116, 1982

Hastings, R.C. and J.R. Trautman. Anti-leprosy agents. Pharmacology in Medicine: Principles and Practice, *Addison-Wesley*, S.N. Pradhan, ed., Chapter 25.4, in press.

Hastings, R.C. The 1982 Journal—A Continuing Perspective. *Int. J. Lep. 51*:89-104, 1983.

Hastings, R.C. and R.R. Jocobson. Activity of Ansamycin against *Mycobacterium leprae* in mice. *Lancet. 2*(8358):1079-1080, 1983.

Hastings, R.C. Release from treatment and follow-up. Health Cooperation Papers, Quaderni di cooperazione sanitaria. *Associazione Italiana Amici di Raoul Follereau 1*:77-79, 1983.

Hastings, R.C. Leprosy. In *Current Therapy in Dermatology, 1985-1986*, T.T. Provost and E.R. Farmer, B.C. Decker, Inc., Philadelphia, Toronto and The C.V. Mosby Company, St. Louis, Toronto, London, pp. 208-212, 1985.

Holzer, T.J., V. Schauf, K.E. Nelson, and B.R. Andersen. Mycobacterial stimulation of human and murine phagocytic cell superoxide anion generation. *Fed. Proc. 43*:1507, 1984.

Holzer, T.J., L. Kizlaitis, V. Schauf, K.E. Nelson and B.R. Andersen. The failure of mycobacteria to stimulate phagocyte superoxide anion generation is correlated with the absence of complement activation *In Vitro*. *J. Leuk. Biol. 36*:204, 1984.

Horwitz, M.A., W.R. Levis, and Z.A. Cohn. Defective production of monocyte activating cytokines in lepromatous leprosy. *J. Exp. Med.*

Horwitz, M.A., W.R. Levis, and Z.A. Cohn. Monocyte activation in leprosy. *J. Exptl. Med.*

Humphres, R.C., R.H. Gelber, and J.L. Krahenbuhl. Natural killer cell activity in patients with lepromatous leprosy. *Fed. Proc. 40*:1044, 1981.

Humphres, R.C., R.H. Gelber, and J.L. Krahenbuhl. Suppressed natural killer cell activity during episodes of erythema nodosum leprosum in lepromatous leprosy. *Clin. Exp. Immunol. 49*:500-508, 1982.

Humphres, R.C., R.H. Gelber, and J.L. Krahenbuhl. Effector function of mononuclear cells from leprosy patients. *Int. J. Lep. 49*:514-515, 1981.

Humphres, R.C., R.H. Gelber, and J.L. Krahenbuhl. Natural killer cell activity in patients with lepromatous leprosy. *Clin. Exp. Immunol. 49*:500-508, 1982.

Hunter, S.W. and P.J. Brennan. A novel phenolic glycolipid from *Mycobacterium leprae* possibly involved in immunogenicity and pathogenicity. *J. Bacteriol. 147*:728-735, 1981.

Hunter, S.W., T. Fujiwara and P.J. Brennan. Structure and antigenicity of the major specific glycolipid antigen of *Mycobacterium leprae*. *J. Biol. Chem. 257*:15072-15078, 1982.

Hunter, S.W. and P.J. Brennan. Further specific extracellular phenolic glycolipid antigens and a related diacylphthiocerol in *Mycobacterium leprae*. *J. Biol. Chem. 258*:7556-7562, 1983.

Imaeda, T., W.F. Kirchheimer and L. Barksdale. DNA isolated from *Mycobacterium leprae*: genome size, base ratio, and homology with other related bacteria as determined by optical DNA-DNA reassociation *J. Bacteriol. 150*:414-417, 1982.

Imaeda, T., L. Barksdale, and W.F. Kirchheimer. Deoxyribonucleic acid of *Mycobacterium lepraemurium*: its genome size, base ratio and homology with those of other mycobacteria. *Int. J. Syst. Bacteriol. 32*:456-458, 1982.

Jacobs, W.R., J.E. Clark-Curtiss, L.R. Ritchie, and R. Curtiss III. Construction and partial characterization of *Mycobacterium leprae* genomic libraries using an *in vivo* cosmid cloning system. *Int. J. Lep. 51*:667–668, 1983.

Job, C.K. Lepromin test and its role in the management of leprosy. *Ethiopian Med. J. 21*:233–242, 1983.

Job, C.K. Leprosy—the source of infection and its mode of transmission. *Lep. Rev.* (Suppl.) *52*:69–76, 1981.

Job, C.K. Leprosy Vaccine—a puzzle. Editorial. *Leprosy in India 55*:1–7, 1983.

Job, C.K. and C.J.G. Chacko. A simplified six group classification of leprosy. *Leprosy in India 54*:26–32, 1982.

Job, C.K., S. Chehl, and R.C. Hastings. Bacilliary clearance in a lepromatous nude mouse following an injection with hepatitis virus. *Leprosy in India 55*:177–178, 1983.

Job, C.K., S. Chehl, R.C. Hastings, and J.R. Ruby. Foam—the result of an interaction between unactivated macrophages and dead *M. leprae*. *Int. J. Lep. 51*:286–287, 1983.

Job, C.K., W.F. Kirchheimer, and R.M. Sanchez. Experimentally produced borderline leprosy in an armadillo. *Int. J. Lep. 50*:488–493, 1982.

Job, C.K., W.F. Kirchheimer, and R.M. Sanchez. Tissue response to lepromin, an index of susceptibility of armadillo to *M. leprae* infection—a preliminary report. *Int. J. Lep. 50*:177–182, 1982.

Job, C.K., R.M. Sanchez, and R.C. Hastings. Attempts to breed the nine-banded armadillo *(Dasypus novemcinctus)* in captivity—a preliminary report. *Int. J. Lep. 52*:362–364, 1984.

Job, C.K., R.M. Sanchez, and R.C. Hastings. Manifestations of experimental leprosy in the armadillo. *Am. J. Trop. Med. Hyg.*, 1985, in press.

Job, C.K., R.M. Sanchez, and W.F. Kirchheimer. Variable lepromin response to *M. leprae* in resistant armadillo. *Int. J. Lep. 51*:347–353, 1983.

Job, C.K., S. Chehl, J.R. Ruby, and R.C. Hastings. Invasion of liver parenchymal cells by *M. leprae* in an experimentally infected nude mouse. An electron microscopic study. *Am. J. Trop. Med. Hyg. 32*:1088–1095, 1983.

Johnson, F.B., Y. Fukunishi, G.P. Walsh, W.M. Meyers, C.H. Binford, P.J. Gerone, R.H. Wolf, B.J. Gormus, and L.N. Martin. Electron microscopic study of leprosy in a mangabey monkey—Naturally acquired infection. *Int. J. Lep. 52*:, 1984, in press.

Kaplan, G., W.C. Van Voorhis, E.N. Sarno, N. Nogueira, and Z.A. Cohn. The cutaneous infiltrates of leprosy. A translation electron microscopy study. *J. Exp. Med. 158*:1145–1159, 1983.

Kaplan, G. and Z.A. Cohn. Hansen's Disease as a research model. In *Bulletin of the New York Acad. Med. 60*:712–721, 1984.

Krahenbuhl, J.L. and R.C. Humphres. The effects of the presence of intracellular *Mycobacterium leprae* on macrophage microbicidal and cytotoxic capacity. *Fed. Proc. 40*:1075, 1981.

Krahenbuhl, J.L. and R.C. Humphres. Effects of treatment with *Proprionibacterium acnes* on growth of *Mycobacterium leprae* in the mouse footpad. *Infect. Immun. 37*:183, 1982.

Krahenbuhl, J.L., R.C. Humphres, and R.W. Ferraresi. Effects of adjuvant immunotherapy on infection with *M. leprae* and *M. marinum*. *Int. J. Lep. 48*:504–505, 1980.

Krahenbuhl, J.L. and R.C. Humphres. Effects of footpad infection with *Mycobacterium marinum* and *M. leprae* on natural killer cell activity to local infection. *Int. J. Lep. 49*:512, 1981.

Krahenbuhl, J.L., R.C. Humphres, and P.C. Henika. Effects of *Propionibacterium acnes* treatment on the course of *Mycobacterium leprae* infection in mice. *Infect. Immun. 37*:183–188, 1982.

Krahenbuhl, J.L. and R.C. Humphres. Effects of treatment with muramyl dipeptide on resistance to *Mycobacterium leprae* and *Mycobacterium marinum* infection in mice. *Immunopharmacology 5*:329–330, 1983.

Kvach, J.T., M.G. Munguia, and S.H. Strand. Staining tissue-derived *Mycobacterium leprae* with fluorescein diacetate and ethidium bromide. *Int. J. Lep. 52*:176–182, 1984.

Kvach, J.T. and J.R. Veras. A fluorescent staining procedure for determining the viability of mycobacterial cells. *Int. J. Lep. 50*:183–192, 1982.

Leininger, J.R., K.J. Donham, and W. Meyers. Leprosy in a chimpanzee: Postmortem lesions. *Int. J. Lep. 48*:414–421, 1980.

Leininger, J.R., K.J. Donham, M.J. Rubino, and W.M. Meyers. Naturally acquired leprosy in a chimpanzee: Necropsy findings and experimental transmission to other animals. *Lab. Invest. 42*:36, 1980.

Levis, W.R., J.S. Schuman, S.M. Friedman, and S.A. Newfield. An epidemiologic evaluation of leprosy in New York City. *J. Am. Med. Assn. 247*:3221–3226, 1982.

Levis, W.R. Treatment of leprosy in the United States. *Bull. NY Acad. Med. 60*:696–711, 1984.

Lovik, M., F.M. Collins, and O. Closs. Inbred C3H mouse substrain differences demonstrated in experimental murine leprosy. *Immunogenetics 16*:607-611, 1983.

Martin, L.N., B.J. Gormus, R.H. Wolf, G.B. Baskin, P.J. Gerone, W.M. Meyers, G.P. Walsh, H.L. Brown, C.H. Binford, C.J. Schlagel, and T.L. Hadfield. Immunological effects of experimental leprosy in the mangabey *(Cerocebus atys) Int. J. Lep. 51*:665-666, 1983.

Martin, L.N., B.J. Gormus, R.H. Wolf, G.P. Walsh, W.M. Meyers, C.H. Binford, and M. Harboe. Experimental leprosy in nonhuman primates. In *Advances in Veterinary Science and Comparative Medicine, Research on Nonhuman Primates.* Academic Press, Inc., New York, NY. *28*: 201-236, 1984.

Martin, L.N., B.J. Gormus, R.H. Wolf, P.J. Gerone, W.M. Meyers, G.P. Walsh, C.H. Binford, T.L. Hadfield, and C.J. Schlagel. Depression of lymphocyte responses to mitogens in mangabeys with disseminated experimental leprosy. *Cellular Immunity*., in press.

Martin, L.N., B.J. Gormus, and B.E. Bozelka. Functional analysis of monkey lymphocyte subsets defined by OKT4 and OKT8 monoclonal antibodies. *Cell. Immunol. 77*:338-347, 1983.

Matsuo, Y., H. Tatsukawa, J.F. Murray, Jr., and J.H. Peters. Prothionamide and prothionamide-S-oxide in experimental leprosy. *Int. J. Lep. 49*:302-306, 1981.

McAdam, K.P.W.J., D. Mudd, Y. Schoenfeld, R.J.W. Rees, D. Draper, D.B. Stollar, and R.S. Schwartz. Mechanisms of auto-antibodies production in leprosy. *Clin. Res. 31*:452, 1983.

Mehra, V., L.H. Mason, W. Rothman, E. Reinherz, S.F. Schlossman, and B.R. Bloom. Delineation of human T-cell subset responsible for lepromin-induced suppression in leprosy patients. *J. Immunol. 125*:1183-1188, 1980.

Mehra, V., J. Convit, A. Rubinstein, and B.R. Bloom. Activated suppressor T cells in leprosy. *J. Immunol 123*:1946-1951, 1982.

Mehra, V., P.J. Brennan, E. Rada, J. Convit, and B.R. Bloom. Lymphocyte suppression in leprosy induced by a unique *M leprae* glycolipid. *Nature 308*:194-196, 1984.

Meier, J., D.S. Folse and J.H. Smith. Leprosy in Wild Armadillos *(Dasypus novemcinctus)* of the Texas Gulf Coast. Ultrastructure of liver and spleen. *Lab. Invest. 49*(3):281-290, 1983.

Meyers, W.M., G.P. Walsh, C.H. Binford, H.L. Brown, R.H. Wolf, B.J. Gormus, L.N. Martin, and P.J. Gerone. Multibacillary leprosy in unaltered hosts, with emphasis on armadillos and monkeys. *Int. J. Lep. 50*:584-585, 1982.

Meyers, W.M., G.P. Walsh, C.H. Binford, R.H. Wolf, B.J. Gormus, L.N. Martin, P.J. Gerone, and G.B. Baskin. Experimental leprosy in three species of monkeys. *Lab. Invest 50*:40A, 1984.

Meyers, W.M., C.H. Binford, G.P. Walsh, R.H. Wolf, B.J. Gormus, L.N. Martin, and P.J. Gerone. Animal models of leprosy. In *Microbiology, 1984*. Washington, DC: American Society for Microbiology, 307-311, 1984.

Meyers, W.M., G.P. Walsh, C.H. Binford, G.D. Imes, Jr., P.J. Gerone, R.H. Wolf, B. J. Gormus, and L.N. Martin. Leprosy in mangabey monkeys—Naturally acquired infection and transmission studies. *Int. J. Lep. 52*: 1984, in press.

Meyers, W.M. Leprosy. In *Textbook of Pediatric Infectious Diseases*. Feigin and Cherry, eds. Philadelphia: W.B. Saunders Co., pp. 880-895, 1981.

Meyers, W.M. Mycobacterial infections of the skin. In *International Textbook of Medicine*. (Volume II: *Medical Microbiology and Infectious Diseases)*. Braude, ed. Philadelphia: W.B. Saunders Co., pp. 1596-1610, 1981.

Meyers, W.M. Early recognition of leprosy. In *New Developments in Tropical Medicine II*. Thomas W. Simpson, G. Thomas Strickland, and Mary Ann Mercer, eds. Washington, DC: National Council for International Health, p. 77, 1983.

Meyers, W.M. Leprosy. In *Hunter's Tropical Medicine*, 6th edition. G. Thomas Strickland, ed. Philadelphia. W.B. Saunders Co., pp. 409-422, 1984.

Meyers, W.M. Mycobacterial infections of the skin. In *International Textbook of Medicine*. (Volume II. *Medical Microbiology and Infectious Diseases*, 2nd edition. Braude, ed. Philadelphia. W.B. Saunders, Co., pp. 1669-2687, 1984.

Meyers, W.M., C.H. Binford, H.L. Brown, and G.P. Walsh. Leprosy in Wild Armadillos. In *Proceedings of a Symposium on the Comparative Pathology of Zoo Animals*, Smithsonian Institution Press, Washington, DC, pp. 247-251, 1980.

Meyers, W.M., D.H. Connor, L. Bevans, and R. Terry. Case for Diagnosis: Lepromatous Leprosy in Traumatically Ruptured Spleen. *Military Medicine 145*:472-481, 1980.

Meyers, W.M., G.P. Walsh, C.H. Binford, H.L. Brown, P.J. Gerone, and R.H. Wolf. Leprosy in the "sooty" mangabey monkey *(Cercocebus torquatu atys)*: Clinical, histopathologic and passage studies. *Lab. Invest. 46*:56A, 1982.

Meyers, W.M., G.P. Walsh, C.H. Binford, R.H. Wolf, B.J. Gormus, L.N. Martin, P.J. Gerone, and G.B. Baskin. Comparative Pathology of Disseminated Multibacillary Leprosy. *Comp. Pathol. Bull. XVI*, 2:1, 5, 6, May 1984.

Meyers, W.M., G.P. Walsh, H.L. Brown, C.H. Binford, G.D. Imes, Jr., T.L. Hadfield, C.J. Schlagel, Y. Fukunishi, P.J. Gerone, R.H. Wolf, B.J. Gormus, L.N. Martin, M. Harboe, and T. Immaeda. Leprosy in a mangabey monkey—Naturally acquired infection. *Int. J. Lep. 53*: 1985, in press.

Miller, R.A., S. Dissanayake and T.M. Buchanan. Development of an enzyme-linked immunosorbent assay using arabinomannan from *Mycobacterium smegmatis*: A potentially useful screening test for the diagnosis of incubating leprosy. *Am. J. Trop. Med. Hyg.* 32:555–564. 1983.

Miller, R.A. and T.M. Buchanan. Production and characterization of a murine monoclonal antibody recognizing a shared mycobacterial polysaccharide. *Int. J. Lep.* In press. Dec. 1984.

Miller, R.A., J.P. Harnish, and T.M. Buchanan. Antibodies to mycobacterial arabinomannan in leprosy: correlations with reactional states during treatment. *Int. J. Lep.* 52:133–140. June 1984.

Modlin, R.L., F.M. Hofman, C.R. Taylor, and T.H. Rea. T-lymphocyte subsets in the skin lesions of patients with leprosy. *J. Am. Acad. Dermatol.* 8:182–189. 1983.

Modlin, R.L., F.M. Hofman, P.R. Meyer, O.P. Sharma, C.R. Taylor, and T.H. Rea. *In situ* demonstration of T-lymphocyte subsets in granulomatous inflammation: leprosy, rhinoscleroma and sarcoidosis. *Clin. Exp. Immunol.* 51:430–438. 1983.

Modlin, R.L., J.F. Gebhard, C.R. Taylor, and T.H. Rea. *In situ* characterization of T-lymphocyte subsets in the reactional states of leprosy. *Clin. Exp. Immunol.* 53:17–24. 1983.

Modlin, R.L., S.A. Vacarro, A.C. Bakke, D.A. Horwitz, C.R. Taylor, and T.H. Rea. T-lymphocyte subpopulations in skin and blood of patients with erythema nodosum leprosum. *Arch. Dermatol.* V. In Press.

Modlin, R.L., G. Rowden, C.R. Taylor, and T.H. Rea. Comparison S-10 and OKT-6 immunostaining patterns in skin lesions from patients with leprosy. *J. Invest. Dermatol.* 83:206–209. 1984.

Modlin, R.L., F.M. Hofman, D.A. Horwitz, L.A. Husmann, S. Gillis, C.R. Taylor, and T.H. Rea. *In situ* identification of cells in human leprosy granulomas with monoclonal antibodies to interleukin-2 and its receptor. *J. Immunol.* 132:3085–3090. 1984.

Mohagheghpour, N., D.T. Sasaki, P.J. Brennan, E.G. Engleman, and R.H. Gelber. Effects of recombinant IL2 on the *Mycobacterium leprae*-induced T cell response in lepromatous leprosy. *Int. J. Lep.*, in press.

Morrison, N.E. and F.M. Collins. Antimycobacterial activity of 2-acetylpyridine thiosemicarbazones in relation to their antileprosy activity. *Int. J. Lep.* 49:180–186. 1981.

Moschella, S.L. Leprosy. In *Dermatology*, 2nd edition. S.L. Moschella and H.J. Hurley (eds.), W.B. Saunders, Philadelphia, PA. pp. 946–972. 1984.

Moschella, S.L. Leprosy Today. *Australian Journal of Dermatology* 24:47–54. 1983.

Murray, K.A., B.A. McLelland, and C.K. Job. Early leprosy with perineural proliferation. *Arch. Dermatol.* 120:360–361. 1984.

Murray, J.F., Jr., G.R. Gordon, J.H. Peters, J. Grove, and G.P. Walsh. Metabolic disposition of dapsone, rifampin and prothionamide in nine-banded armadillos *(Dasypus novemcinctus)*. In Leprosy. F. Latapi et al., eds., *Excerpta Medica*, Princeton, NJ. p. 227. 1980.

Navalkar, R.G. Immunology of Leprosy. *CRC Critical Reviews of Microbiology.* 8: 1980.

Navalkar, R.G., P.J. Patel, and M.V. Kanchana. Studies on immune response to *Mycobacterium lepraemurium*. Evaluation of the cell-mediated immune response in mice. *Int. Arch. Allergy.* 63:423. 1980.

Navalkar, R.G. and M.V. Kanchana. Immune response to *Mycobacterium lepraemurium*. Assessment of the humoral immune response in mice. *Zbl. Bakt. Infekt. Hyg.* 248:110. 1981.

Navalkar, R.G. Immune response to *Mycobacterium leprae*. Further studies on the assessment of humoral immune response in mice. *Zbl. Bakt. Infekt. Hyg.* 247:364. 1980.

Navalkar, R.G., S.D. Chaparas, C.K. Lakshiminarayana, and M.V. Kanchana. Antigenic evaluation of *Mycobacterium vaccae. Int. J. Lep.* 48:388. 1980.

Nelson, K.E. and V. Schauf. Response to "Risks of treating leprosy in a general hospital." *Int. J. Lep.* 51:413–416. 1983.

Nelson, K.E., S.M. Speck, S. Suprasert, and T. Smith. A Study of Cellular Immunity in Clinically Healthy Children of Parents with Leprosy in Northern Thailand. *Int. J. Lep.* 52:147–153. 1984.

Neylan, T., K.E. Nelson, V. Schauf, and D.M. Scollard. Explanatory Models of Leprosy in Patients in Northern Thailand. In *Culture, Medicine and Psychiatry*, in press.

Nogueira, N., G. Kaplan, E. Levy, E.N. Sarno, P. Kushner, A. Granelli-Piperno, L. Vieira, V.C. Gould, W. Levis, R. Stinman, Y.K. Yip, and Z.A. Cohn. Defective-IFN production in leprosy: reversal with antigen and interleukin-2. *J. Exptl. Med.* 158:2165–2170. 1983.

Peters, J.H., G.R. Gordin, W. Tanaka, and V.F. Simmon. Mutagenesis-carcinogenesis of antileprosy drugs in animal systems: pertinence to man. In *Leprosy*. F. Latapi et al., eds., Excerpta Medica, Princeton, NJ. p. 230. 1980.

Peters, J.H., R.H. Gelber, R.R. Jacobson, A.B.G. Laing, and M.F.R. Waters. Tissue levels of dapsone during combined dapsone-rifampin therapy. In *Leprosy*. F. Latapi et al., eds., Excerpta Medica, Princeton, NJ. pp. 221–226. 1980.

Peters, J.H., J.F. Murray, Jr., G.R. Gordon, and R.H. Gelber. Dapsone in saliva and plasma of man. *Pharmacology* 22:162–171. 1981.

Peters, J.H., K.J. Hamme, and G.R. Gordon. Determination of clofazimine in plasma by high-performance liquid chromatography. *J. Chromatog.* 229:503–508. 1982.

Peters, J.H., G.R. Gordon, J.F. Murray, Jr., and V.F. Simmon. Mutagenic activity of antileprosy drugs and their derivatives. *Int. J. Lep.* 51:45–53. 1983.

Peters, J.H., G.R. Gordon, and J.F. Murray, Jr. Disposition of prothionamide in rats and armadillos. *Int. J. Lep. 51*:54–63, 1983.

Poulter, L.W., L.A. Collings, K.S. Tung, and F.R. Waters. Parasitism of antigen presenting cells in hyperbacillary leprosy. *Clin. Exp. Immunol.* 55:611-617, 1984.

Prabhakaran, K. Acetylsalicyclic acid and senile cataract. *IRCS Med. Sci.* 9:928, 1981.

Prabhakaran, K. Is diphenoloxidase a constitutive enzyme in *Mycobacterium leprae? Lep. India* 54:832-833, 1982.

Prabhakaran, K., W.F. Kirchheimer, R. Sanchez, and E.B. Harris. An alternative route for infecting armadillos with *Mycobacterium leprae. Microbios. 39*:83-86, 1984.

Prabhakaran, K., E.B. Harris, S. Tsutsumi, and W.F. Kirchheimer. Effect of fusaric acid on *o*-diphenoloxidase and on multiplication of *Mycobacterium leprae. Microbios Letters* 22:81-84, 1983.

Prabhakaran, K., E.B. Harris, and W.F. Kirchheimer. Effect of purification procedures on viability of *Mycobacterium leprae. Int. J. Lep. 48*:330-331, 1980.

Prabhakaran, K., E.B. Harris, and W.F. Kirchheimer. Failure to detect *o*-diphenoloxidase in cultivable mycobacteria obtained from feral armadillos. *Lep. Rev. 51*:341-349, 1980.

Prabhakaran, K., E.B. Harris, and W.F. Kirchheimer. Glutamic acid decarboxylase in *Mycobacterium leprae. Arch. Microbiol 134*:320-323, 1983.

Prabhakaran, K., E.B. Harris, and W.F. Kirchheimer. Permeability of *Mycobacterium leprae* to dapsone: alteration by purification procedures. *Lep. India 53(2)*:160-162, 1981.

Prabhakaran, K., E.B. Harris, and W.F. Kirchheimer. A possible method for improving the efficacy of dapsone. *Experientia 36*:1350-1351, 1980.

Prabhakaran, K., and E.B. Harris. Temperature-induced changes in viability, diphenoloxidase and permeability of *Mycobacterium leprae. Microbios*, 1985, in press.

Ramos-Caro, F.A. "Histoid Lepromas." Presented at the meeting at the Puerto Rico Dermatological Society, Dorado, Puerto Rico. *Cutis 30*: 1980.

Ramsey, P.R., D.F. Tyler, J.R. Waddill, and E.E. Storrs. Blood chemistry and nutritional balance of wild and captive armadillos *(Dasypus novemcinctus* L.). *Comp. Biochem. Physiol 69A*:517-521, 1981.

Rea, T.H., P.I. Terasaki. HLA-DR antigens in tuberculoid and lepromatous leprosy. *Lep. Rev. 51*:117-123, 1980.

Rea, T.H., and N.E. Levan. Variations in dinitrochlorobenzene responsivity in untreated leprosy: evidence for a beneficial role for anergy. *Int. J. Lep. 48*:120–125, 1980.

Rea, T.H. and C.R. Taylor. The Histopathology of the spleen from a patient with lepromatous leprosy. *Int. J. Lep. 48*:290, 1980.

Rea, T.H., and T. Yoshida. Serum macrophage migration inhibition activity in patients with leprosy. *J. Invest. Dermatol 79*:336-339, 1982.

Rea, T.H., R.B. Narayanan, and J.L. Turk. Lysozyme and angiotensin-converting enzyme levels in experimental mycobacterial granulomas. *J. Path. 139*:207-216, 1983.

Rea, T.H. Suppressor cell activity and phenotypes in the blood or tissues of patients with leprosy. *Clin. Exp. Immunol. 54*:298-304, 1983.

Rea, T.H., A.C. Bakke, J.W. Parker, R.L. Modlin, and D.A. Horwitz. Peripheral blood T-lymphocyte subsets in leprosy. *Int. J. Lep. 52*:311-317, 1984.

Ridel, P.-R., J.S. Johl, and J.L. Krahenbuhl. Enhanced local cell mediated immunity in mice treated with macrophages harboring *Mycobacterium leprae. Fed. Proc. 42*:861, 1983.

Ridel, P.R., J.S. Johl, and J.L. Krahenbuhl. Cell mediated immunity in mice treated with *Mycobacterium leprae* or with macrophages harboring *M. leprae. Institute Pasteur Annals d'Immunol. 135*:39, 1984.

Ridley, D.S., T.H. Rea, and K.P.W.J. McAdam. The histology of erythena nodosum leprosum. Variant forms in New Guineans and other ethnic groups. *Lep. Rev. 52*:65-78, 1981.

Ridley, J. and C.K. Job. Pathology of Leprosy. In *Textbook of Leprosy*. Edited by R.C. Hastings, in press.

Ridley, D.S., T.H. Rea, and K.P.W.J. McAdam. Variations in the histology of erythema nodosum leprosum. *Lep. Rev. 52*:65-78, 1981.

Sarno, E.N., G. Kaplan, F. Alvaranga, N. Nogueira, J.A. Porto, and Z.A. Cohn. The effect of treatment on the cellular composition of cutaneous lesions in leprosy patients. *Int. J. Lep.* 1984, in press.

Schauf, V., K.E. Nelson, and W.E. Bullock. The immunobiology and epidemiology of leprosy. A collaborative project between universities of the USA and Thailand. *Lep. Rev. 53(2)*:151-153, 1982.

Schauf, V. East-West efforts key into leprosy research. *JAMA 51*:15-18, 1984.

Schroff, R.W., E.R. Heise, and Q.N. Myrvik. Characterization of the Early Phase of the Tuberculin Reactions in Rabbits. *Am. Rev. Respir. Dis. 121*:843-850, 1980.

Schroff, R.W., E.R. Heise, Q.N. Myrvik, and B.T. Shannon. Selective Suppression of Granuloma Formation and Delayed Hypersensitivity in Rabbits. *Infect. Immun. 28*:269-276, 1980.

Shannon, E.J., M. Powell, R.R. Jacobson, and R.C. Hastings. Investigations of suppressor mechanisms in clinical leprosy. *Dermatology Revista Mexicana* 25:457–468, 1981.

Shannon, E.J., M.D. Powell, W.F. Kirchheimer, and R.C. Hastings. Effects of *M. leprae* antigens on the *in vitro* responsiveness of mononuclear cells from armadillos to Concanavalin-A. *Lep. Rev.* 55:19–31, 1984.

Shannon, E.J., R.O. Miranda, M.J. Morales, and R.C. Hastings. Inhibition of *de novo* antibody synthesis by thalidomide as a relevant mechanism of action in leprosy. *Scand. J. Immunol.* 13:553–562, 1981.

Shepard, C.C., R. Van Landingham, and L.L. Walker. Searches among mycobacterial cultures for antileprosy vaccines. *Infect. Immun.* 29:1034–1039, 1980.

Shepard, C.C., P. Draper, R.J.W. Rees, and C. Lowe. Effect of purification steps on the immunogenicity of *Mycobacterium leprae*. *Brit. J. Exp. Path.* *61*:376–379, 1980.

Shepard, C.C., F. Minagawa, R. Van Landingham, and L.L. Walker. Foot pad enlargement as a measure of induced immunity to *Mycobacterium leprae*. *Int. J. Lep.* *48*:371–381, 1980.

Shepard, C.C. *Mycobacterium leprae*. In *The Prokaryotes; A Handbook on Habitats, Isolation and Identification of Bacteria*. M.P. Starr, ed., Springer, New York, pp. 1985–1990, 1981.

Shepard, C.C. A brief review of experiences with short-term clinical trials monitored by mouse-foot-pad inoculation. *Lep. Rev.* 52:299–308, 1981.

Shepard, C.C. Statistical analysis of results obtained by two methods for testing drug activity against *Mycobacterium leprae*. *Int. J. Lep.* *50*:96–101, 1982.

Shepard, C.C., L.L. Walker, R.M. Van Landingham, and S-Z. Ye. Sensitization or tolerance to *Mycobacterium leprae* antigen by route of injection. *Infect. Immun.* *38*:673–680, 1982.

Shepard, C.C. Leprosy Today. *N. Eng. J. Med.* *307*:1640–1641, 1982.

Shepard, C.C., R.M. Van Landingham, L.L. Walker, and S-Z. Ye. Comparison of the immunogenicity of vaccines prepared from viable *Mycobacterium bovis* BCG, heat-killed *Mycobacterium leprae*, and a mixture of the two for normal and *M. leprae*-tolerant mice. *Infect. Immun.* *40*:1096–1103, 1983.

Shepard, C.C., R.M. Van Landingham, and L.L. Walker. Recent studies in antileprosy drugs. *Lep. Rev.* *54*:235–305, 1983.

Shepard, C.C. Animal vaccination studies with *Mycobacterium leprae*. *Int. J. Lep.* *51*:519–523, 1983.

Shepard, C.C., D.L. Klayman, J.P. Scovill, and N.E. Morrison. 2-Acetylpyridine thiosemicarbazones and *Mycobacterium leprae*. *Int. J. Lep.* 52:7–9, 1984.

Shepard, C.C. Immunity to leprosy and the Mitsuda reaction (Editorial). *Int. J. Lep.* 52:74–77, 1984.

Smith, J.H., D.S. Folse, E.G. Long, J.D. Christie, D.T. Crouse, M.E. Tewes, A.M. Gatson, R.L. Ehrhardt, S.K. File, and M.T. Kelly. Leprosy in wild armadillos *(Dasypus novemcinctus)* of the Texas gulf coast: Epidemiology and mycobacteriology. *J. Ret. Soc.* *34*:75–88, 1983.

Stoner, G.L., J. Touw, and A. Belehu. An assay for antibodies in leprosy sera reacting with ribonucleoprotein (RNP), a mycobacterial ribosomal antigen, using crossed immunoelectrophoresis with intermediate gel. *Int. J. Lep.* *48*:247–253, 1980.

Stoner, G.L., R.N. Mshana, J. Touw, and A. Belehu. Studies on the defect in cell-mediated immunity in lepromatous leprosy using HLA-D-identical siblings: Absence of circulating suppressor cells and evidence that the defect is in the T lymphocyte, rather than the monocyte population. *Scand. J. Immunol.* *15*:33–48, 1982.

Stoner, G.L., T. Atlaw, J. Touw, and A. Belehu. Antigen-specific suppressor cells in subclinical leprosy infection. *Lancet*:1372–1377, 1981.

Stoner, G.L., A. Belehu, J. Nsibambi, and J. Warndorff. Borderline tuberculoid leprosy following BCG vaccination. A case report. *Int. J. Lep.* *49*:16–20, 1981.

Stoner, G.L. Hypothesis: Do phases of immunosuppression during a *Mycobacterium leprae* infection determine the leprosy spectrum? *Lep. Rev.* 52:1–10, 1981.

Storrs, E.E. The astonishing armadillo. *National Geographic Magazine* *161*:820–830, 1982.

Storrs, E.E., and H.P. Burchfield. Leprosy in wild common long-nosed armadillos *Dasypus novemcinctus*. In *The Evolution and Ecology of Armadillos, Sloths and Vermilinguas*. G.G. Montgomery, ed. Smithsonian Institution Press, 1984, in press.

Storrs, E.E. Armadillos. In *UFAW Handbook on the Care and Management of Laboratory Animals*. T.B. Poole, ed. United Fed. for Animal Welfare Press, 1984, in press.

Trautman, J.R., W.F. Kirchheimer, K. Prabhakaran, R.C. Hastings, E.J. Shannon, R.R. Jacobson, and P.W. Brand. An overview of Carville research. *Acta Leprologia* *84*:1–29, 1981.

Turkel, S.B., H.M. Van Hale, T.H. Rea. Ultrastructure of the dermal microvasculature in leprosy. *Int. J. Lep.* *501*:164–171, 1982.

Van Hale, H.M., S.B. Turkel, T.H. Rea. Dermal ultrastructure in leprosy. *Arch. Pathol. Lab. Med.* *108*:383–386, 1984.

Van Voorhis, W.C., G. Kaplan, E.N. Sarno, M.A. Horwitz, R.M. Steinman, W.R. Levis, N. Nogueira, L.S. Hair, C.R. Gattas, B.A. Arrick, and Z. Cohn. The cutaneous infiltrates of leprosy. Cellular characteristics and the predominant T-Cell phenotypes. *New Eng. J. Med. 307*:1593-1597, 1982.

Walsh, G.P., W.M. Meyers, C.H. Binford, H.L. Brown, R.H. Wolf, B.J. Gormus, L.N. Martin, G.B. Baskin, and P.J. Gerone. Experimental leprosy in the rhesus monkey and the African green monkey. *Int. J. Lep. 52*: 1984, in press.

Walsh, G.P., W.M. Meyers, C.H. Binford, R.H. Wolf, B.J. Gormus, L.N. Martin, P.J. Gerone, and J.R. Leininger. Leprosy as a zoonosis (Exhibit). *Int. J. Lep. 52*: 1984, in press.

Walsh, G.P., W.M. Meyers, and C.H. Binford. Animal Models in the Evaluation of Chemotherapy of Leprosy. In *Animal Models in the Evaluation of Chemotherapy of Infectious Diseases*. O. Zak and M.A. Sande, eds. London, Academic Press, Inc. (London) Limited. Two volumes, 1985, in press.

Walsh, G.P., W.M. Meyers, C.H. Binford, P.J. Gerone, R.H. Wolf, and J.R. Leininger. Leprosy—A Zoonosis. *Lep. Rev. 52* (Suppl. 1):77-83, 1981.

Watson, S.R. and W.E. Bullock. Immunoregulatory Disturbances in Leprosy. In *Host Defenses to Intracellular Pathogens*, eds., T.K. Eisenstein, P. Actor and H. Friedman, Plenum Publishing Corp., NY, 1983.

Watson, S.R., W.E. Bullock, K.E. Nelson, R. Schauf, R. Gelber, and R.R. Jacobson. Interleukin 1 production by peripheral blood mononuclear cells from leprosy patients. *Infect. Immun. 45*:787-789, 1984.

Watson, S.R., W.E. Bullock, K.E. Nelson, V. Schauf, R. Gelber, and R.R. Jacobson. Studies on interleukin 1 production by peripheral blood mononuclear cell from leprosy patients. *Int. J. Lep. 51*:649-650, 1983.

Welch, T.M., R.H. Gelber, L.P. Murray, H. Ng, S. O'Neill and L. Levy. Viability of *Mycobacterium leprae* after multiplication in mice. *Infect. Immun. 30*:325, 1980.

Wilt, T.J., C.M. Buys, P.J. Gudewicz, B.R. Andersen, V. Schauf, and K.E. Nelson. Failure of *Mycobacterium leprae* to stimulate macrophage post phagocytic chemiluminescence and glucose oxidation. *Clin. Research 30*:4, 778A, 1982.

Wolf, R.H., L.N. Martin, B.J. Gormus, G.B. Baskin, P.J. Gerone, G.P. Walsh, W.M. Meyers, H.L. Brown, and C.H. Binford. Experimental transmission of leprosy in African green monkeys *(Cercopithecus aethiops)* and the rhesus monkey *(Macaca mulatta)*. *Int. J. Lep. 51*:664-665, 1983.

Wolf, R.H., B.J. Gormus, L.N. Martin, G.B. Baskin, G.P. Walsh, W.M. Meyers, and C.H. Binford. Experimental leprosy in three species of monkeys. *Science*. December 1984, in press.

Young, D.B., and T.M. Buchanan. A serological test for leprosy using a glycolipid specific for *Mycobacterium leprae*. *Science 221*:1057-1059, 1983.

Young, D.B., S.R. Khanolkar, L.L. Barg, and T.M. Buchanan. Generation and characterization of monoclonal antibodies to the phenolic glycolipid of *Mycobacterium leprae*. *Infect. Immun. 43*:183-188, 1984.

Young, D.B., S. Dissanayake, R.A. Miller, S.R. Khanolkar and T.M. Buchanan. Humans respond predominantly with IgM immunoglobulin to the species specific glycolipid of the leprosy bacillus. *J. Infect. Dis. 149*:870-873, 1984.

Joint Scientific Conferences

LEPROSY PANELS

Date: October 9–11, 1980

Place: Kagoshima, Japan

Summary: The 15th Joint Conference on Leprosy Research was held in Kagoshima, Japan, under the sponsorship of the U.S.-Japan Cooperative Medical Science Program. Highlights included a number of papers on experimental animal models of leprosy including athymic nude mice, neonatally thymectomized Lewis rats, congenitally athymic nude rats, asplenic mice, naturally infected and experimentally infected armadillos, and for the first time naturally acquired leprosy in a mangabey monkey. Drug studies emphasized high performance liquid chromatographic drug measurement techniques, studies of chemotherapy in nude mice and clinical primary sulfone resistant leprosy. Seroepidemiologic studies suggested for the first time that infection with *M. leprae* was relatively common among individuals in a leprosy-endemic area, but that actual disease signs or symptoms were relatively rare. Early work was reported on protein antigenic analysis of *M. leprae*. Refinements were made in cultivation media for *M. lepraemurium*. As in all U.S.-Japan Leprosy Research Conferences, the manuscripts were published in the Conference Proceedings and the abstracts of the papers were published in the International Journal of Leprosy.

Date: July 13–14, 1981

Place: NIH, Bethesda, Maryland

Summary: The 16th Joint Conference was held in Bethesda. Electron-microscopic and biochemical studies of the peribacillary substance of *M. leprae* were reported and for the first time an apparently unique phenolic glycolipid was described in *M. leprae*. Circumstantial evidence pointed to this phenolic glycolipid as a major component of the peribacillary substance which has long been recognized around *M. leprae in situ*. The intact mouse was shown to be capable of detecting persister leprosy organisms. A fluorescent staining technique was described to show viability of *M. leprae*. Measurements of ATP were used to estimate viability of *M. leprae*. Lepromatous leprosy patients were shown to have impaired immunoregulatory control over their B lymphocyte function.

Date: August 25–27, 1982

Place: Sendai, Japan

Summary: The 17th Joint Leprosy Conference was held in Sendai, Japan. Improvements in techniques for *in vitro* cultivation of *M. lepraemurium* were reported. It was speculated that *M. leprae* may divide *in vivo* once every 26 hours based on only the live bacilli rather than the traditional 2 weeks based on the total number of bacilli. A variety of experimental animal models of lepromatous leprosy were described including armadillos, primates, nude rats, and nude mice. Serologic responses to the phenolic glycolipid antigen of *M. leprae* were described as well as more detailed antigenic analysis of protein antigens of the leprosy bacillus. Characteristic patterns of T subset distributions in the skin lesions of lepromatous and tuberculoid leprosy patients were described. Helper and suppressor T cells were randomly distributed in lepromatous lesions but in tuberculoid lesions the central portion of the "tubercle" was made up of phenotypically helper T cells adjacent to epithelioid cells surrounded by a mantle of predominantly suppressor T cells.

Date: August 3–5, 1983

Place: NIH, Bethesda, Maryland

Summary: The 18th Joint Leprosy Conference was held at the same time as the 18th Joint U.S.-Japan Tuberculosis Conference with a combined leprosy-tuberculosis session on the final day. Mice intravenously infected with 10^7 *M. leprae* were shown to be tolerant to subsequent immunization with 10^7 organisms intradermally. BCG was capable of reversing this immunologic tolerance but did not induce protection against challenge with viable *M. leprae*. An increased number of helper/inducer T cells and high helper/suppressor ratios found in patients with erythema nodosum leprosum (ENL) suggested that cell-mediated immune responses might be important in the pathogenesis of ENL, either in permitting production of an antibody critical to immune complex formation or directly as a cell-mediated immune response. *M. leprae*-infected nude mice developed reversal reactions after being administered spleen cells from immunocompetent nu/+ heterozygotes. Measurements of bacillary ATP were applied to leprosy patients undergoing chemotherapy. Nineteen murine monoclonal antibodies were described against *M. leprae*. Nine were directed against the phenolic glycolipid antigen of *M. leprae*, 8 were directed against a 68,000 dalton protein and 1 each to arabinomannan and a 14,000 dalton protein. Promising results were obtained in field seroepidemiologic studies measuring IgM antibody responses to the phenolic glycolipid antigen of *M. leprae*. Among feral armadillos in Texas, 4.7% had leprosy. Both rhesus and African green monkeys, but not squirrel monkeys, were shown to be susceptible to experimental leprosy although not as consistently susceptible as mangabey monkeys. The construction of several kinds of mycobacterial recombinant DNA libraries in *E. coli* (including *M. leprae*) were described but no evidence was found at that time that the proteins expressed by these DNAs were functional. Active, synthetic, *M. leprae*-specific antigens were chemically synthesized based on the terminal disaccharide of the phenolic glycolipid antigen.

Date: August 27–29, 1984

Place: Tokyo, Japan

Summary: The 19th Joint Conference was held in Tokyo, Japan. An approach to cultivation of *M. leprae* was described based on plasmid-mediated gene transfer from *M. intracellulare*. In leprosy skin lesions interleukin-2 (IL-2) producing cells were ten-fold more abundant in BT and ENL lesions than in lepromatous lesions without ENL. In contrast, the number of anti-Tac positive cells were similar in all three tissues. This suggests that antigen has been presented to, and IL-1 has been produced and recognized by, helper T cells in lepromatous leprosy. Because IL-2 producing T cells are reduced in lepromatous leprosy lesions, the basic failure of specific cell-mediated immunity in lepromatous leprosy would therefore seem to be due to reduced, perhaps inhibited, IL-2 production. The "normal" number of IL-2 producing T cells in ENL can be interpreted as evidence that ENL is a cell-mediated immune response, and the deinhibition of IL-2 production is important in its pathogenesis. Serum IgM antibodies against the phenolic glycolipid antigen of *M. leprae* were reported to be linearly related to bacterial load in patients and to be significantly lower in patients with ENL than in lepromatous patients without ENL, suggesting that these antibodies may be involved in the pathogenesis of ENL. Routine lepromin skin testing was shown to significantly increase the positivity of the fluorescent leprosy antibody absorption test in normal individuals. Recombinant IL-2 and "conditioned medium" augmented the *in vitro* proliferative activity of peripheral blood lymphocytes from all types of leprosy patients (and from healthy individuals) to an equivalent degree and regardless of the presence or absence of antigenic stimuli. Thus, there was no evidence that IL-2 specifically restores the response of most patients with lepromatous leprosy to *M. leprae* antigens. Further work with synthetic specific antigens of *M. leprae* showed 2–3% "false-positive" seroreactivities against this moiety of the phenolic glycolipid antigen of *M. leprae* among healthy individuals from non-leprosy endemic areas. More than 90% of biopsy proven new cases of leprosy from endemic areas were found to be serologically positive prior to the onset of disease. A group of murine monoclonal antibodies were characterized. In all, these recognized 8 antigenic molecules of *M. leprae*, 6 of these molecules contained epitopes which were apparently specific for *M. leprae*—5 of these being proteins and 1 being the phenolic glycolipid antigen.

MALNUTRITION PANEL

Drs. [illegible] Suzue and Irwin Rosenberg, Chairmen
of the Malnutrition Panel, U.S.-Japan Cooperative
Medical Science Program

Dr. [illegible] Suzue (Chairman, 1982)
Director
National Institute of Nutrition
1-23-1, Toyama, Shinjuku-ku
Tokyo 162, Japan

Dr. Irwin H. Rosenberg, Chairman (1981–), Member (1975–81)
Professor of Medicine
Head, Section of Gastroenterology
The Pritzker School of Medicine
950 East 59th Street
Chicago, Illinois 60637

PANEL MEMBERS

Japan

Dr. Fumimaro Takaku (1982–)
Professor
Department of Internal Medicine
Faculty of Medicine
Tokyo University
7-3-1, Hongo, Bunkyo-ku
Tokyo 113, Japan

Dr. [illegible] (19[illegible])
Professor
Department of Medicine
Faculty of Medicine
Osaka University
4-3-57, Nakanoshima, Kita-ku
Osaka 530, Japan

Dr. [illegible] (19[illegible])
Professor
Department of Physiology
Keio University School of Medicine
35, Shinanomachi, Shinjuku-ku
Tokyo 160, Japan

United States

Dr. James D. Cook (1979–)
Professor
Department of Hematology
University of Kansas Medical Center
Kansas City, Kansas 66103

Dr. James A. Olson (1981–)
Chairman, Department of Biochemistry and Biophysics
Iowa State University
Ames, Iowa 50011

Dr. Barbara Underwood (1974–)
Special Assistant for Nutrition Research
National Institutes of Health
National Eye Institute
Building 31, Room 6A-[illegible]
Bethesda, Maryland 20205

Dr. Gumpei Urata (1984-)
Director
Department of Nutritional Biochemistry
Institute of Public Health
4-6-1, Shirokanedai, Minato-ku
Tokyo 108, Japan

Former Members

Dr. Tatsuo Abe (1979-1982)
Director
Yokohama-shi Nanbu Hospital
3-3-1, Konandai, Konan-ku
Yokohama 233, Japan

Dr. Tadataka Fukui (1976-1981)
Director
Institute of Arteriosclerosis
Health and Welfare Association
3-19-7, Toranomon, Minato-ku
Tokyo 105, Japan

Dr. Goro Inoue, Chairman (1978-1984)
Professor
Nakamura-Gakuen University
5-7-1, Beppu, Jonan-ku
Fukuoka 814, Japan

Dr. Tetsuzo Takahashi (1976-1984)
Professor
Department of Nutrition
Institute of Health and Sport Science
Tsukuba University
Tennodai, Sakura-mura, Shiji-gun
Ibaraki Prefecture 305, Japan

Dr. Hiroshi Wako (1977-1982)
Director
Hachinohe Japan Red Cross Hospital
2, Tamonogi Nakaakito, Hachinohe-shi
Aomori Prefecture 031, Japan

Former Members

Dr. Frank T. Falkner (1978-1983)
University of California
School of Public Health
Maternal and Child Health
Earl Warren Hall
Berkeley, California 94720

Dr. Sheldon Margen, Chairman (1978-1983, Member 1971-1978)
Professor of Public Health
Head of Public Health Nutrition Program
School of Public Health
University of California
Berkeley, California 94720

Japanese Malnutrition Panel

GOALS AND OBJECTIVES

The overall goal of the Malnutrition Panel is to explore scientific measures that could lead to eradication of malnutrition in the world. Today, malnutrition is induced not only by undernutrition, but also by overnutrition.

To prevent diseases caused by malnutrition, both the U.S. and Japanese panels have agreed to accept the following five priority areas as cogent research objectives:

1) Determination of the effects of nutrition on physical and mental development, behavior, physical capacity, and work performance
2) Studies of health significance and methods of prevention of nutritional anemia
3) Influence of environmental and host factors on requirements for health in Asian countries
4) Malnutrition due to metabolic disorders
5) Health consequences of different and changing dietary patterns and food habits

The first four research objectives are directed at undernutrition or unbalanced nutrition. To help prevent such abnormal nutrition, improvement in food distribution, economic development, increase in food production and in nutrition education are also necessary.

Research related to overnutrition is only in the area of the fifth objective and this objective applies to overnutrition all over the world. The research objectives of this Panel are not fixed, but will be changed according to socio-economic factors and to new research needs and opportunities.

PROGRESS IN PRIORITY AREAS

Determination of the effects of nutrition on physical and mental development, behavior, physical capability, and work performance

Unfavorable effects of malnutrition on mental development have been thought to be a very important research field. Malnutrition was found to result in abnormal metabolism of nutrients and in inhibition of dendritic growth. Protein deficiency caused strong inhibition of fetal development and decreased weight of uterus and placenta. In pregnant and non-pregnant rats fed on a protein-free diet, cellular DNA quantity and liver cell size were remarkably diminished. When abundant protein was provided to pregnant rats, normal growth rate of brain cells was restored.

No statistically significant differences were found in anthropometrical values between breast fed and formula fed infants. However, from a psychological, behavioral, and immunological standpoint, breast feeding showed superiority to formula milk feeding. It was concluded that breast feeding is best for human infants.

As iron deficiency is still one of the most serious problems in developing countries, investigations on the influence of deficiency in iron and other trace elements upon mental development are now under way.

Studies of health significance and methods of prevention of nutritional anemia

A most important objective is development of methods for easier detection of marginal anemia. Marginal anemia is found by measurement of hemoglobin, serum iron, serum transferrin saturation, serum ferritin, and free erythrocyte protoporphyrin (FEP), most importantly by protoporphyrin in the red blood cell and serum ferritin. Using these indices, about 10% of aged Japanese seemed to be suffering iron deficiency. These methods, however, are very expensive and complicated, so more convenient methods must be explored.

To prevent iron deficiency, fortification of iron in food is most effective, but iron fortified foods are frequently unpalatable because of peroxide formation from fats in foodstuffs. Thus, the palatability of biscuit, mayonnaise, and "Tsukudani" fortified with various amounts of iron was examined. Among these, "Tsukudani," dried anchovy boiled down in soysauce, was most favorable. Ferrous sulfate was used as the fortifier so that 20g of "Tsukudani" contained 75mg of iron. Significant increases in hemoglobin, hematocrit, and serum iron were observed in women receiving daily 10 to 20g of "Tsukudani" for 3 months.

Influences of environmental and host factors on requirements for health in Asian countries

Effects of normal and low protein intakes (1.3g/kg and 0.5g/kg) on urea reutilization were investigated in Papua New Guinea highlanders, who are adapted to low protein intake, and in Japanese men. 15N-urea was utilized for plasma protein synthesis in both groups. The extent of 15N incorporation into plasma protein in the Highlanders receiving standard protein was similar to that on low protein intake. The results were not the same, however, in the Japanese controls.

The requirements of essential amino acids for rat growth were determined by feeding for 14 days 10% amino acid diets simulating the amino acid pattern of egg. In these studies the dietary amino acid concerned was varied among 6 to 11 levels. Regression equations between body protein gain and intake of amino acid concerned showed that requirements (mg) per gram of protein gain were 50.3 for Ile, 89.6 for Leu, 71.6 for Lys, 48.4 for Met, 88.9 for Phe, 39.3 for Thr, 16.9 for Trp, 51.7 for Val, 63.2 for Arg, and 25.4 for His. These values were close to those of the NRC/NAS.

To estimate the protein allowances of low-birth-weight infants, effects of three milk formulae (moderate protein-fat, low protein-high fat and relatively high protein-fat) on growth, fat absorption, and protein retention were investigated for 4 weeks after birth. Approximate weight gains were 35g/day and 40g/day during the 3rd and 4th weeks respectively, and there were no differences among the dietary groups.

Effects of exercise on dermal nitrogen losses and urinary nitrogen excretion were examined in 5 young men in connection with protein and energy intakes. Dermal nitrogen loss increased during the periods of exercise or of high protein intake with moderate energy intake, whereas urinary excretion of nitrogen did not change.

Differences in serum cholesterol and fat intake by age, district, and occupation were observed. Average cholesterol levels and fat intake were higher in urban areas than in rural areas; there was little difference, however, in teenagers. Compared with data of Americans, average cholesterol levels were lower in adults, but little difference was observed in teenagers. P/S ratio in adults was higher in rural areas than in urban areas.

Eicosapolyenoic acids in serum from aged Japanese on Iriomote and Kohama Islands in Okinawa prefecture were analyzed to determine why they have a low incidence of cardiovascular disease. The acid levels were higher than those of the controls on the mainland, presumably due to the abundant intake of fresh fish. In addition, a positive correlation between eicosapentanoic acid levels and HDL-cholesterol in serum was found.

Lipid concentrations in serum from 10,977 Japanese subjects were measured in 1980, and the values were compared with those measured in 1961 and 1970. Average figures (mg/100 ml) were about 190 for total cholesterol, 114 and 98 for triglyceride in males and females, respectively, and about 55 for HDL-cholesterol. Due to a two-fold increase in fat intake and a 1.6 increase in protein intake, total cholesterol in 1980 was elevated by around 15 mg/100 ml over 1961. Results indicate that serum lipids in the Japanese are changing to higher levels slowly but steadily.

ACHIEVEMENT OF MALNUTRITION PANEL DURING THESE 20 YEARS

In 1966, the U.S. and Japan Malnutrition Panels began by identifying specific research areas which could significantly influence programs for the prevention of malnutrition in the developing countries of Asia. At that time, influenced by undernutrition, infectious diseases were highly prevalent in Asia and in the whole world, and the average life expectancy was also extremely short. This situation was thought to be related to the shortage of energy, protein, vitamin A, iron, and iodide. The Panels started to explore scientific measures that could lead to the eradication of malnutrition all over the world.

The priority research areas for the Japanese Malnutrition Panel were as follows:

1. Malnutrition and mental development
2. Nutritional anemias
3. Evaluation of nutritional status and nutritional requirements
4. Nutritional adaptation
5. Evaluation of new protein sources

In research on mental development and learning ability, the implication of early malnutrition on growth and development and on later learning and behavior was explored. Deficiencies of protein, folate, and vitamin C were clearly found to affect brain development. Comparative studies revealed higher morbidity and mortality in bottle-fed infants than in breast-fed infants. Artificial nursing of newborn cynomolgus monkeys was used as a model for human infants in studies of development of abnormal behavior. The effect of malnutrition on the growth of the brain during infancy was studied. From psychological, behavioral, and immunological standpoints, breast feeding showed superiority to mild formula feeding.

Regarding the relationship between anemia and some nutrients, it was found that anemia occurred in cases of folate, iron, and protein deficiencies. To explain the relationship between pregnancy and anemia, standardization of blood levels was necessary.

In the reports on the evaluation of nutritional status and nutritional requirements, protein nutrition was mainly studied. As an index of protein deficiency, the determination of the plasma protein ratio was most important. The plasma amino acid ratio was found to have a significant correlation with weight gain, dietary protein level, and hydoxyproline index. Availability of ingested protein may be greatly affected by the protein sparing action of ingested energy. Accordingly, the maintenance N requirement should be measured under the condition of maintenance energy intake which exactly meets individual energy expenditure. In the course of the studies of new protein sources, the effects of lysine and threonine fortification of rice in Japanese rural areas and lysine fortification of bread in school lunches were reported.

The above reports were all related to undernutrition, and they were all reasonable and necessary investigations under the specific social condition. The results were all useful for recommendations on breast-milk feeding and school lunches. After the increase in world food supply and the consequent extended life expectancy, research on overnutrition became necessary even in Southeast Asia. For these reasons we changed the Japanese name of the Panel to "EIYO-IJO Panel," meaning

Nutritional guidance for aged people

areas as follows:

1. Effects of nutrition on mental development
2. Health significance of iron deficiency.
3. Nutritional requirements in Asian countries
4. Nutrition and infection.
5. Health consequences of different food habits

These five priority areas were established in 1978

Today it is necessary to elucidate the relationships between overnutrition and adult health and diseases. By this research the effects of salt and fat on cardiovascular diseases are being clarified; for example, fish oil containing w-3 fatty acids has been found to be very effective in improving the patterns of serum lipids. Viewpoints of nutritional research are annually changing, and the Malnutrition Panel is now interested in taking up the problem of overnutrition in relation to cardiovascular disease.

FUTURE GOALS

The Malnutrition Panel has two major research objectives: undernutrition and overnutrition. In the past, research has concentrated on undernutrition, but now world-wide economic development and improvement in cultivation has produced adequate food supplies, except in parts of Africa. At the same time, life expectancy is increasing remarkably. The application of nutrition to prevention of cardiovascular diseases now is becoming important even in Asia.

In order to meet the needs of this new situation, we are planning to propose five new important priority research areas as

areas, all Japanese panel members were changed in 1983 and 1984, and new investigators have been appointed. The new priority areas are as follows:

1. Health consequences of different dietary patterns and food habits for the aged in the different parts of Japan, the United States, and Asia, especially the effects of differences in calcium intake on bone health of the elderly.
2. Development of new methods for nutrition assessment. In the world, including Asia, there have been no standard parameters of anthropometry because of the differences of race, physiological states, and food patterns. Development of new evaluating methods and of new equipment for measurement is important.
3. Nutritional effect of polyunsaturated fatty acids for prevention of adult diseases. The intake of polyunsaturated fatty acids is mainly from vegetable oil in the U.S. and from fish oil in Japan and Asia. W-3 fatty acids in fish oil may be very effective against arteriosclerosis and coronary heart disease. Nutritional and epidemiological studies on fish oil are important.
4. Sulfur-containing amino acids. Glutathione and taurine have a close relationship to metabolism of polyunsaturated fatty acids. Vitamin B6 is also an important factor in sulfur-containing amino acid metabolism.
5. Nutrition and prevention of diseases, especially of cancer and infectious diseases. Comparative studies between Japan and the U.S. are needed to clarify the relationship between nutritional status and endogenous mediators, for example interleukin, in prevention of viral, bacterial, and parasitic infections.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
Yoshiaki Niiyama University of Tokushima	Effect of malnutrition during pregnancy on fetal growth in rats
Tomoichi Kusunoki Kyoto Prefectural University of Medicine	Nutrition and brain
Hiroshi Wako Iwate Medical University	Determination of the effects of malnutrition on physical and mental development, behavior, physical capability, and work performance
Yasuzo Tsukada Keio University	Abnormal development of the brain and malnutrition
Tomiji Hatakeyama Iwate Medical University	Determination of the effects of malnutrition on physical and mental development, behavior, physical capability, and work performance
Taro Inoue Kobe Women's University	Studies on anemia in human subjects
Akio Urabe University of Tokyo	Erythropoiesis and nutritional factors

Keizo Shiraki University of Occupational and Environmental Health	Nutritional anemia
Fumimaro Takaku University of Tokyo	Effect of iron deficiency on infection and immunity
Shigeru Nomura Kumamoto University	Community study on control of iron deficiency anemia in women
Hiroshi Furuya Juntendo University	Studies on iron metabolism and iron deficiency in pregnancy
Tadataka Fukui National Institute of Nutrition	Methods of prevention of nutritional anemia
Tetsuzo Takahashi University of Tsukuba	Effect of exercise on protein metabolism
Shuhei Kobayashi National Institute of Nutrition	Health state and nutrient intake of aged people of Japan
Ryokuero Suzue National Institute of Nutrition	Effects of nutrients on plasma lipoprotein metabolism
Yasutoshi Muto Gifu University	Nutritional oncology of fat-soluble vitamins
Toshio Yamada Kyoto Technical University of Industrial Arts and Textile Fibers	Studies on protein metabolism and hard muscular work in relation to its nutrition requirement
Yoshiro Wada Nagoya City University	Inborn errors of metabolism, nutritional disturbances and treatment
Hideo Koishi Osaka City University	Protein nutrition in Papua New Guinea highlanders
Kyoichi Kishi University of Tokushima	Studies on protein and amino acid requirements
Takuo Fujita Kobe University	Calcium and aging
Tetsuro Morita Kawasaki Medical School	Nutritional availability of several kinds of formulae in infant nutrition
Hiroyuki Toyokawa University of Tokyo	Epidemiological studies on both dietary pattern and thickness of subcutaneous fat measured by A-mode ultrasonics
Yoshio Komachi University of Tsukuba	Epidemiology and community prevention of cerebro- and cardiovascular diseases
Masashige Suzuki University of Tsukuba	Nutritional consequences of feeding patterns
Yuichiro Goto Tokai University	Influence of Japanese food patterns and food habits on lipid metabolism
Tatsuo Abe Saiseikai Yokohamashi Nanbu Hospital	Influences of nutritional effect of different nutrients or dietary habit on health
Tohru Abei Toho University	Studies on the nutritional state of patients with acute diarrhea

BEST [illegible] LE

BIBLIOGRAPHY

Abe. T. National nutrition survey in Japan proceeding Methodologies for Human Population Studies in Nutrition Related to Health. NIH Publication No. 82-2462:142-148. September. 1982. U.S.A

Abe. T. A relapse of beriberi in Japan. *ibid.*. 185-192

Abe. T. Nutrition and diseases. *Nutrition and Food 30*:337-344. 1977

Abe. T. The old and vitamins. *Geriat. Med.* 22:19-24. 1984

Abe. T. Studies of beriberi in Japan. *Nutrition and Food 30*:323-328. 1977

Abe. T. The role on the pathogenesis and clinical application of protein and amino acid. *Nutrition and Food*. 27:245-253. 1974

Abei. T. et al. Biliary diseases in Bolivia. *J. Med. Soc. Toho* 28:809-818. 1981

Abei. T. et al. A clinical study on gallstones in Bolivia. *Jap J. Med.* 22:90-94. 1983

Esashi. T., R. Suzue and J. Leathem. Influence of dietary protein depletion and repletion on sex organ weight of male rats in relation to age. *J. Nutr. Sci. Vitaminol.* *28*:163-172. 1982

Fujita. T., M. Fukase. Y. Yoshimoto. M. Tsutsumi. T. Fukami. Y. Imai. K. Sakaguchi. T. Abe. M. Sawai. I. Seo. T. Yaguchi. S. Enomoto. D.M. Droke. and L.V. Avioli. Basic and clinical evaluation of measurement of bone resonant frequency. *Calif Tissue Int.* *35*:153-158. 1983

Fujita. T. Aging of connective tissue and disease. *Connective Tissue*. 16(1):19-24. 1984.

Fujita. T., Y. Okamoto. Y. Sakagami. K. Ota. and M. Ohata Bone changes and aortic calcification in aging inhabitants of mountain versus seacoast communities in the Kii Peninsula. *J Amer. Geriat. Soc. 32(2)*:124-128. 1984.

Fujita. T., H. Baba. Y. Yoshimoto. M. Fukase. M. Kishihara. M. Tomon. T. Fukami. Y. Imai. Y. Nishii. and M. Fukushima Physiological significance of PTH degradation through limited hydrolysis by rat kidney cytosolic enzymes. Pp. 238-241. Endocrine control of bone and calcium metabolism. D.V. Cohn. T. Fujita. et al ed. Elsevier Science Publishers B.V., 1984.

Fujita. T. Serum calcium—an important determinant of the survival of elderly patients. *Kobe J. Med. Sci.* 30:1-6. 1984

Fujita. Y., T. Rikimaru. T. Okuda. C. Date. N. Kajiwara. K Yanase and H. Koishi. Studies on nutrition of Papua New Guinea highlanders: Balance and hematological studies. *J. Nutr Sci. Vitaminol.* *28*:431. 1982.

Fujita. Y., T. Okuda and H. Koishi. Studies on nitrogen balance and endogenous nitrogen excretion in highlanders of Papua New Guinea. Reports of the Research Committee of Essential Amino Acids. *Jap. 101*:23. 1984

Fukui. T. Studies on prophylactive effects of nutritional anemia Reports from National Institute of Nutrition. 1978

Furuya. H. Nutritional control of anemic pregnant women *Obstetrics & Gynecology Series*. *23*:213-219. 1979

Furuya. H., M. Kurabayashi. K. Kokuho. and H. Hirayama Therapeutic directions for anemia in pregnancy—New trend due to introduction of serum ferritin determination. *Obstetrical & Gynecological Therapy*. *39*:20-25. 1979

Futatsuka. M. et al. Follow-up studies on the distribution of hemoglobin levels in female farm workers. *J. Epidemiol. Community Health*. *33*:286. 1979

Futatsuka. M. et al. Studies on the seasonal variation of blood properties in farmers. *J. J. P. H.* *27*:211. 1980.

Futatsuka. M. et al. Hemoglobin values in adult women of various occupational groups in the community. *Jap. J. Ind Health*. *26*:155. 1984

Goto. Y. Atherosclerosis and its risk factors in Japan *Atherosclerosis*. 5:703-706. Springer-Verlag. 1980

Goto. Y. Hyperlipidemia and atherosclerosis in Japan *Atherosclerosis*. *36*:341 1980

Goto. Y. Clinical diagnosis of atherosclerosis. *J. Jap. College Angiol.* *21*:225. 1980

Goto. Y. Effects of alpha- and beta-blocker: Antihypertensive therapy on blood lipido. Multicenter trial. *Amer. J. Medicine 76*:72. 1984.

Goto. Y. Genetic and environmental factors for longevity. *Jap J. Geriatrics*. *21*:34. 1984

Hatakeyama. T. Experimental child care—Monkey psychiatry *The Journal of Pediatric Practice*. *Jap. 46(1)*:16-20. 1983

Hatakeyama. T. Long-term effect of mother-infant separation in cynomolgus monkeys. *Perinatal Medicine*. *13(12)*:53-59. 1983

Hatakeyama. T. Obesity in childhood. *The Japanese Journal of Clinical Nutrition*. *63(1)*:13-18. 1983

Hatakeyama. T Monkey psychiatry as a model of human *Pediatric Review* *16(1)*:134-168. 1983

Hirayama. H., K. Kokuho. M. Kurabayashi. T. Okuyama and H. Furuya. Clinical significances of serum ferritin levels in gynecological diseases with special reference to malignant neoplasms. *Acta Obst. Gynaec. Jap., Kanto Branch. 31*:10–17, 1980.

Hisatomi, K., S. Sasaki and Y. Niiyama. Effect of maternal protein and/or energy deficiency during pregnancy on catecholamines and serotonin in fetal rat brain. *J. Nutr. Sci. Vitaminol. 25*:243–253, 1979.

Hisatomi, K. and Y. Niiyama. Effect of postnatal undernutrition on the catecholamine and serotonin contents of suckling rat brain. *J. Nutr. Sci. Vitaminol. 26*:279–292, 1980.

Igarashi, Y., F. Yokota, R. Suzue and S. Chikaraishi. The effects of megadoses of ascorbic acid on lipid metabolism in rats and guinea pigs. *Vitamins. Jap. 56*:91–95, 1982.

Inoue, G., T. Komatsu, K. Kishi, and Y. Fujita. Amino acid requirements of Japanese young men *in* "Amino acids, metabolism and medical applications," ed. by Blackburn, G.L., J.P. Grant, V.R. Young, and John Wright PSG Inc. Boston 55–62, 1983.

Kajimoto, M., M. Kondo, M. Niwa, T. Suzuki, H. Kimura, A. Sasaki and G. Urata. Increase of -aminolevulinic acid dehydratase (ALAD) in rat erythrocytes in lead poisoning. *Arc Toxicol. 52*:1–11, 1983.

Kishi, K., K. Rikimaru, Y. Matsumoto, F. Shizuka, and G. Inoue. Constancy of net protein intake in rats on self-selection regimen with proteins of various qualities. *Jpn. J. Physiol. 32*:959–970, 1982.

Kishi, K., G. Inoue, Y. Yoshimura, S. Yamamoto, and T. Yamamoto. Quantitative interrelationship between effects of nitrogen and energy intakes on egg protein utilization in young men. *Tokushima J. Exp. Med. 30*:17–24, 1984.

Kitamura, K., A. Urabe, K. Ozawa, Y. Kimura, and F. Takaku. Enhancement of human T lymphocyte colony formation by 12-O-tetradecanoylphorbol-13-acetate (TPA). *Exp. Hematol. 11*:1014, 1983.

Koba, F. Blood properties and growth of school children. *J. J. P. H. 31*:257, 1984.

Kobayashi, S. Diet and diseases: Heart infarction. *Diagnosis and Therapy. 72(10)*:2027, 1984.

Kohsaka, S., and Y. Tsukada. Neurochemical and behavioral studies on the experimental phenylketonuric rats. *Keio Journal of Medicine. 28*:97–108, 1979.

Kohsaka, S., K. Takamatsu, and Y. Tsukada. Effect of food restriction on serotonin metabolism in rat brain. *Neurochemical Research. 5(1)*:69–79, 1980.

Koishi, H., T. Okuda, H. Miyoshi, Y. Fujita, M. Ichikawa, N. Kajiwara, and S. Miyatani. Reutilization of urea with N-urea on Papua New Guinea highlander. Reports of The Research Committee of Essential Amino Acids. *Jap. 97*:58, 1983.

Kokuho, K., M. Kurabayashi, T. Okuyama, and H. Furuya. Clinical observation of serous ferritin levels in obstetrics and gynecology—Relation between maternal, fetal, and neonatal sera. Act. Obst. Gynaec. *Jpn. 32(11)*:1775–1782, 1980.

Komachi, Y., et al. Multivariate analysis of risk factors for stroke eight-year follow-up study of farming villages in Akita, Japan. *Preventive Medicine. 9(6)*:722–740, 1980.

Komachi, Y., T. Shimamoto, M. Iida and M. Konishi. Recent changes of environmental factors and trends of stroke in Japan. *Japanese Journal of Stroke. 3(2)*:82, 1981.

Komachi, Y. et al. Dietary intake and serum total cholesterol level: Their relationship to different lifestyles in several Japanese populations. *Circulation. 66(3)*:519–526, 1982.

Komachi, Y. et al. Pathological study on stroke relating to change in life style in Akita. *Japanese Journal of Public Health 29(3)*:105, 1982.

Komachi, Y. et al. A collaborative study of stroke incidence in Japan 1975–1979. *Stroke. 15(1)*:28–36, 1984.

Komatsu, T., K. Kishi, T. Yamamoto and G. Inoue. Nitrogen requirement of amino acid mixture with maintenance energy in young men. *J. Nutr. Sci. Vitaminol. 29*:169–185, 1983.

Kondo, M., G. Urata, and Y. Shimizu. Decreased liver -amino laevulinate dehydratase activity in porphyria cutanea tarda and in alcoholism. *Clinical Science 65*:423–428, 1983.

Kurabayashi, M. Studies on iron metabolism and iron deficiency anemia in pregnant women-Theoretical discussion on the relation between serum iron level and iron dose to be administered and clinical result after iron therapy. *Acta Obst. Gynaec. J. 32(7)*:833–841, 1980.

Kusunoki, T. The role of central nervous system for occurrence and progress of obesity. *No to Hattatsu. (Tokyo)13*:89, 1981.

Kusunoki, T. Carbohydrate metabolism in livers of mice with monosodium L-glutamate-induced hypothalamic obesity. *J. Chron. Diseas. and Therapeut. Research. 21*:254, 1981.

Kusunoki, T. Effects of early malnutrition and subsequent rehabilitation on brain development-An autoradiographic study. *Brain and Development. 5*:148, 1983.

Kusunoki, T. Effects of early malnutrition and rehabilitation on brain development—Golgi-Cox study. *Brain and Development. 6*:149, 1984.

Lai, A., K.M. Ito, K. Komatsu and Y. Niiyama. Effects of various levels of dietary calcium during pregnancy on maternal calcium utilization and fetal growth in rats. *J. Nutr. Sci. Vitaminol. 30*:285–295, 1984.

Matsueda, S. and Y. Niiyama. The effects of excess amino acids on maintenance of pregnancy and fetal growth in rats. *J. Nutr. Sci. Vitaminol. 28*:557–573, 1982.

Mizoguchi, H., K. Kubota, Y. Miura and F. Takaku. Effect of food deprivation on haemopoietic stem cells. *Biomedicine. 36*:313, 1982.

Morita, T. and S. Kobuchi. Nutritional availability of milk formulae composed of several kinds of protein in infants—Effects on amino acids in plasma and urine. Reports of The Research Committee of Essential Amino Acids. *Jap.* *89*:42–47, 1981

Morita, T. Protein in infant nutrition. Reports of the Research Committee of Essential Amino Acids. *Jap.* *91*:8–12, 1981

Morita, T. and K. Kobayashi. Nutritional availability of taurine supplemented powdered milk formula in infant nutrition. Reports of the Research Committee of Essential Amino Acids. *Jap.* *101*:36–39, 1984

Murata, H. et al. Estudio epidemiologico de la diarrea en el departamento de Cochabamba, Bolivia. *Acta Gastroenterologica Boliviana.* 2:12–16, 1982

Muto, Y. and H. Moriwaki. Antitumor activity of vitamin A and its derivatives. *J. Natl. Cancer Inst.* (in press)

Muto, Y. and M. Omori. A novel cellular retinoid-binding protein, F-type, in hepatocellular carcinoma. *Ann. N.Y. Acad. Sci.* *359*:91–103, 1981

Muto, Y. et al. *In vitro* binding affinity of novel synthetic polyprenoids (polyprenoic acids) to cellular retinoid-binding proteins. *Gann.* *72*:974–977, 1981

Muto, Y., et al. Demonstration of a novel cellular retinoid-binding protein, F-type, in hepatocellular carcinoma. *Gann* *70*:215–222, 1979

Muto, Y. Recent advances in metabolism of vitamin A. *Vitamin* *57*:291–299, 1983

Nishimuta, M., T. Yamada, S. Kobayashi, and T. Suzuki. Effects of exercise and nutrient intake on magnesium balance in human subjects. *J. Jap. Soc. Magnesium Res.* *2(1)*:49, 1983

Noguchi, T., T. Sugisaki and Y. Tsukada. Brain development and growth factors. In "Growth and Growth Factors," ed. by Japan Medical Research Foundation, Univ. Tokyo Press. 203–230, 1980

Okabe, T., M. Fujisawa and F. Takaku. Long-term cultivation and differentiation of human erythroleukemia cells in a protein-free chemically defined medium. *Proc. Natl. Acad. Sci. U.S.A.* *81*:453, 1984

Okasaki, S., S. Matsueda, M. Ohnaka, and Y. Niiyama. Effect of various periods of protein restriction immediately after weaning on subsequent catch-up growth in rats. *Nutr. Rep. Int'l* *23*:471–484, 1981

Okuda, T., N. Kajiwara, C. Date, K. Sugimoto, T. Rikimaru, Y. Fujita, and H. Koishi. Nutrition status of Papua New Guinea highlanders. *J. Nutr. Sci. Vitaminol.* *27*:319, 1981

Sagawa, S. and K. Shiraki. Changes of osmotic fragility of red blood cells due to repletion or depletion of cholesterol in human and rat red cells *in vitro*. *J. Nutr. Sci. Vitaminol.* *26*:161–169, 1980

Saito, T., A. Urabe, K. Uramoto, Y. Hashimoto, and F. Takaku. Serum erythropoietin titres in the anemia of premature infants. *Br. J. Haematol.* *54*:53, 1983

Saito, T., A. Urabe and F. Takaku. *In vitro* assay of erythropoietin: Simple determination in a small amount of human serum samples. *Jap. J. Med.* *23*:16, 1984

Sakai, A. Nutritional availability of milk formulae with different protein compositions in infants. *Acta Paediatrica Japonica* *22(2)*:21, 1980

Shionoya, S., A. Urabe, Y. Hashimoto, M. Kondo and G. Urata. Induction of δ-aminolevulinic acid synthase and δ-aminolevulinic acid dehydratase in normal human bone marrow cultures. *Stem Cells.* 2:145, 1982

Shiraki, K., S. Sagawa, N. Kondo, H. Nakayama and M. Matsuda. Properties of red blood cell after multiday exposure to 31 ATA. *Undersea Biomed. Res.* *10*:349–358, 1983

Shiraki, K., S. Sagawa, N. Kondo and H. Nakayama. Fragility of red blood cells under 31 atmosphere absolute for 14 days. *J. UOEH.* 5:35–46, 1983

Sugimoto, M. et al. Dilatacion congenita de las vias biliares intra y extra hepaticas. *Acta Gastroenterologica Boliviana* *1*:43–49, 1981.

Sugiyama, N., Y. Wada, H. Morishita and I. Nonaka. Different ketogenic response to medium-chain triglycerides and to long-chain triglycerides in a case of muscular carnitine palmitoyltransferase deficiency. *J. Inher. Metab. Dis.* 5:233–234, 1982

Suzuki, M. Effect of voluntary running exercise on hypertriacylglycerolemic effect of sucrose in relation to its feeding timing in rats. *J. Nutri. Sci. Vitaminol.* 29:663, 1983.

Suzuki, M. Diurnal changes in lipolytic activity of isolated fat cells and their increased responsiveness to epinephrine and theophylline with meal feeding in rats. *J. Nutri. Sci. Vitaminol.* 29:399, 1983.

Suzuki, M. Effects of feeding patterns on food intake and body composition of rats. *J. Jap. Soc. Nutr. Food Sci.* *36*:175, 1983.

Suzuki, M. Preventive effects of high iodine intake from iodine-enriched eggs on atherosclerosis, calcification, and decline of thyroid function with aging. *Int. Symp. Nutr. Prevent. Cardiac Diseases.* Kyoto, October, 1983

Suzuki, M. Medical and nutritional surveys in The Kingdom of Tonga: Comparison of physiological and nutritional status of adult Tongans in urbanized (Kolofoou) and rural (Uiha) areas. *J. Nutri. Sci. Vitaminol.* *30*:341, 1984

Takahashi, T. and S. Muramatsu. Effect of dietary protein and energy levels on the changes of nitrogen balance induced by exercise. *Rep. Res. Committee of Essential Amino Acids.* *93*:14, 1982

Takahashi, T. and S. Muramatsu. Effect of exercise on serum free amino acid pattern. *Ibid.* 97:22, 1983

Takahashi, T., S. Muramatsu and Song Ja Hyun. Effect of exercise on leucine requirements in rats. *Ibid.* *100*:46, 1983

Takahashi, T., S. Muramatsu and M. Suzuki. Effect of dietary protein level and voluntary exercise on the morphological and biochemical measurements, and endurance ability of adult rats. Univ. of Tsukuba, *Bull. Health and Sports Sciences.* 7:209, 1984

Takahashi, T. Protein nutrition in physical exercise. *Jap. J. Nutr.* *42*:213, 1984

Takaku, F., T. Suda, H. Mizoguchi, Y. Miura, H. Uchino, K. Nagai, S. Kariyone, A. Shibata, T. Akabane, T. Nomura, and T. Maekawa. Effect of peripheral blood mononuclear cells from aplastic anemia patients on the granulocyte-macrophage and erythroid colony formation in samples from normal human bone marrow *in vitro*: A cooperative work. *Blood.* 55:937, 1980

Takaku, F., T. Suda and Y. Miura. Studies on the pathogenesis of aplastic anemia. *Acta Haematol. Jpn.* *44*:1313, 1981

Takaku, F. Differentiation of hematopoietic cells in hematological disorders. *Acta Haematol. Jpn.* 45:1191, 1982

Tanaka, N., K. Kubo, K. Shiraki, H. Koishi and H. Yoshimura. A pilot study on protein metabolism in the Papua New Guinea highlanders. *J. Nutr. Sci. Vitaminol.* 26:247, 1980

Toyokawa, H. Community nutrition program. *J. Jap. Soc. of Food and Nutrition.* *43(4)*:285-291, 1979

Toyokawa, H. The nutritional status in Japan from the viewpoint of numerical ecology. *Social Science and Medicine* *12(6-A)*:517-524, Pergamon Press, 1978

Toyokawa, H. A study of the relationship between diet and cancer mortality in Japan, *in* "Dietary Influences on Cancer Traditional and Modern," ed. by R. Schoental, T.A. Connors 37-48, CRC Press, 1981

Toyokawa, H., et al. Studies for the practical uses of the A mode type ultrasonic device, (the first report) validity in identification of the target wave at thith. *Jap. J. Public Health* *31(1)*:14-20, 1984

Tsukada, Y. Effect of mild protein restriction during prenatal and postnatal development of rats on discriminative learning ability. In proceeding international nutrition conference, *in* "Behavioral effects of energy and protein deficits," ed. Brozek, J. Published by NIAMDD NIH Publication. 79-1906 12-21, 1979

Tsukada, Y., K. Takamatsu and S. Kohsaka. Changes in serotonin metabolism in the brain of food restricted rats. *Baroda J. Nutr.* 8:78-83, 1981

Urabe, A. and S. Fujioka. Erythroid progenitors in paroxysmal nocturnal haemoglobinuria. *Br. J. Haematol.* *50*:295, 1982

Wada, Y. Inborn errors of nucleic acid metabolism. New Encyclop. of Pediat. and Related Med. *Sci.* *7D*:267-310, Nakayama Book Co., 1982

Wada, Y. Protein metabolism and its regulation. New Encyclop. of Pediat. and Related Med. *Sci.* *17A*:241-253, Nakayama Book Co.

Wada, Y., H. Nakajima, M. Irie, M. Hirayama and S. Suwa. Newborn mass screening in Japan-1984. *Jap. J. Human Genet.* 29:3, 277~, 1984

Wako, H. et al. Nutrition, environment and mental development—Special reference to infant feeding, growth, and development in northern Japan. *Nutrition and Food Science* *3*:709-715, 1980

Wako, H. et al. Reinvestigation in human milk composition. *J. Pediat.* *33(11)*:2207-2214, 1980

Wako, H. Drug doses of deficiency, overadministration, and dependency of vitamins. *J. Pediat.* *34(12)*:3011-3017, 1981

Wako, H. Effects of nutrition and environment on growth and development of fetus and early childhood. *J. Jap. Disposition* *4*:27-30, 1983

Wako, H. History and perspective (Japanese) of child malnutrition. *Shin-Shonikagaku Taikei.* *18*:3-12, 1984

Wang, M.-F., K. Kishi, T. Takahashi, T. Komatsu, M. Ohnaka and G. Inoue. Efficiency of utilization of soy protein isolate in Japanese young men. *J. Nutr. Sci. Vitaminol.* 29:205-216, 1983

Yamada, T. Effect of physical training in a hot environment on heat tolerance of men. *J. Physic. Sci.* 27:3, 100-108, 1978

Yamada, T. Anemia during hard physical training (sports anemia) and its causal mechanism with special reference protein nutrition. *Wld. Rev. Nutr. Diet.* *35*:1-86, 1980 (Karger, Basel)

Yamada, T. Comparison of changes in erythrocyte count, hematocrit, and erythropoietin levels induced by strenuous physical training between athletes and nonathletes. *Jap. J. Phys. Educ.* *25(2)*:119-126, 1980

Yamada, T. Effect of protein nutrition on fetus growth in the pregnant rat. *J.J. Womanhood.* *1*:69-86, 1981

Yamada, T. Comparison of effects of vegetable protein diet and animal protein diet on the initiation of anemia during hard physical training (sports anemia). *Reports of the Research Committee of Essential Amino Acid.* *100*:36-45, 1984

Yamagami, M., H. Takagi, H. Yanai and H. Toyokawa. A study on the international comparison of the pattern of food energy intakes. *Jap. J. Public Health* *26(6)*:307-317, 1979

Yasutake, R. Nutrition surveys in women in agricultural villages and a proposal for the estimate of recommended iron allowance of adult women. *Jap. J. Hyg.* 36:863, 1982.

Yazaki, M., Y. Wada, Y. Kojima, A. Tanaka, G. Isshiki, N. Kawamura and M. Hirose. Copper binding proteins in the liver and kidney from the patients with Menkes' Kinky Hair Disease. *Tohoku J. Exp. Med.* 139:97-102, 1983.

Yokota, F., Y. Igarashi and R. Suzue. Hyperlipidemia in guinea pigs induced by ascorbic acid. *Atherosclerosis.* 38:249-254, 1981.

Yokota, F., Y. Igarashi and R. Suzue. Effects of ethionine feeding on fatty liver and plasma lipoprotein fractions in rats. *J. Nutrition.* 112:405-409, 1982.

Yoshimura, H., T. Inoue, T. Yamada and K. Shiraki. Anemia during hard physical training (sports anemia) and its causal mechanism with special reference to protein nutrition. *World Rev. Nutr. Diet.* 35:1-86, 1980.

U.S. Malnutrition Panel

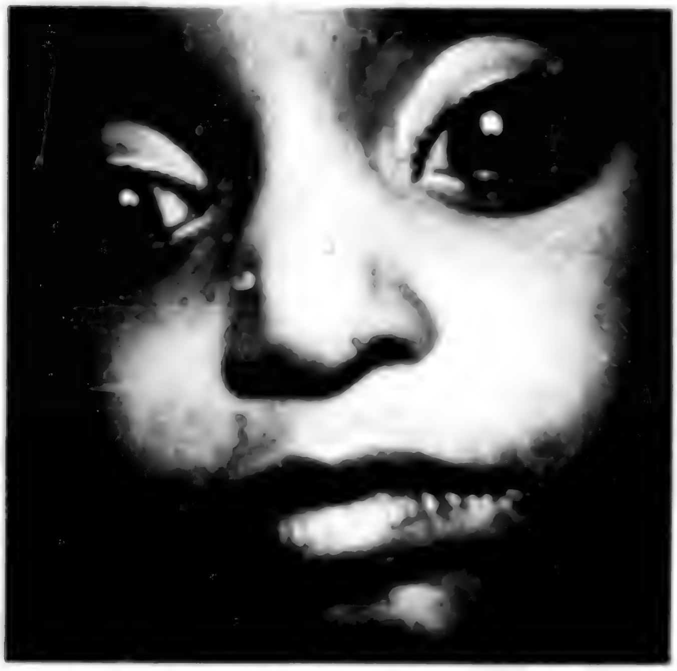
BROAD GOALS AND OVERVIEW

This fourth 5-year report of the U.S. Malnutrition Panel completes the panel's second decade of activity. The years between 1979 and 1984 have witnessed some important changes. After several years of distinguished service, Dr. Sheldon Margen finished his term and turned the chairmanship over to Dr. Irwin Rosenberg. Dr. Gerald Combs, the program officer almost from its inception, changed his position within the U.S. Government and his task as program officer shifted to Dr. Van Hubbard. Dr. Donald Whedon, Director of NIADDK, and one of the guiding spirits of the U.S. panel, retired from that position and the directorship was assumed by Dr. Lester Salans for slightly over 1 year before his departure from the National Institutes of Health.

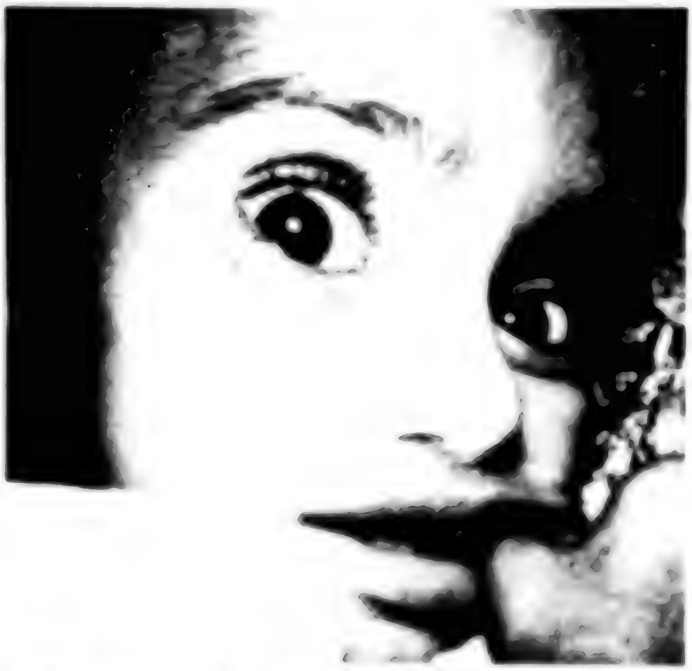
These substantial personnel changes, particularly at the level of the National Institutes of Health, coincided with a searching review of the U.S.-Japan Malnutrition Panel Program within the overall mission of the NIADDK along with a continuing reevaluation of the relationship between the goals of the panel and the resources available to accomplish those goals. This has lead to a review initiated by Dr. Salans, Director of NIADDK, of the scientific opportunities afforded by that program, and this review immediately predated the initiation of the 5-year program review by the U.S.-Japan Committee.

While it is true that the major scientific priority areas (see below) did not change radically over the recent 5-year period, there was a gradual and perceptible shift in emphasis, exemplified by the selection of topics for the yearly scientific workshops, in the direction of an increasing attention to nutritional concerns of the United States and Japan, particularly under the priority area of the health consequences of different and changing dietary patterns and food habits. Inevitably, this shift in emphasis occurred to some extent at the expense of the prior orientation to the nutritional problems of less developed countries in Asia.

In all likelihood the most lasting contributions of the U.S. panel members and their Japanese counterparts during these 5 years will have been the identification, conceptualization, and presentation of a series of yearly workshops which have presented the state of the science and pinpointed research needs in those critical areas relating to the priorities of the U.S.-Japan Malnutrition Panel. These workshops have identified the important active scientists in the United States and Japan, and often in other countries, for presentation of work relevant to the topic of the workshop. Often the scientists selected were not those specifically identified with nutrition but yet had scientific expertise relevant to progress in the field under study. These workshops have been attended by scientists outside the U.S.-Japan Program in addition to the invited participants, and the workshop publications have had a further effect of disseminating important deliberations. Some of these publications have taken the form of a mono[illegible]h and others have taken the form of a supplement to a wi[illegible]ed journal. Usually, the topic of the workshop was one [illegible]pecial interest which would have been difficult to present under other auspices. For instance, the



Child with eye lesions (xerophthalmia) caused by Vitamin A deficiency.



After treatment with Vitamin A.

workshop in Tokyo in December of 1979 on "The Health Implications of Changing Dietary Patterns in the United States and Japan" was a most seminal meeting involving scientists from a broad range of disciplines in both countries. The following workshop in Bethesda which explored the methodologies used in nutrition survey techniques critically and theoretically continues to be a widely used reference source. The workshop on "Vitamin A, Retinoids in Cancer," held in 1983 in Bethesda, was cosponsored by the National Cancer Institute and presented a timely opportunity for scientists from diverse disciplines to meet and identify areas of greatest opportunity.

The members of the panel agree that these workshops and their publications, although a significant contribution in themselves, will only achieve their full potential success when the mechanisms are in place for followup interactions leading to new grants and to new scientific collaboration, especially between American and Japanese scientists.

SUMMARY OF THE THIRD FIVE YEARS

From 1975 through 1980 the objectives of the panel remained essentially the same as during the earlier periods. The influence of the environment and host factors on nutritional requirements for health and the effects of nutritional status on physical and mental development, behavior, physical capacity, and work performance were areas which received increased attention during this 5-year period. Originally, the emphasis had been on the potential effects on mental development of chronic malnutrition of the mother and infant or child. During recent years, increased attention has been placed on the physical development and work performance associated with malnutrition. In spite of intensive research, new questions continue to arise. Studies indicate little doubt that severe protein malnutrition leads to impairment of mental development. Studies were initiated to determine whether the mechanism involved is a function of biochemical alterations, growth retardation, or social factors. Along with the development of new technologies, as well as the awareness of the importance of trace mineral metabolism, numerous studies investigating the effect of trace mineral nutriture were initiated. Particular attention was paid to studies of zinc and copper.

The increased emphasis on trace mineral metabolism also carried over into the priority area of the interaction of nutrition with immune competence and infection. Studies to determine the immunological capacity of malnourished individuals to respond to natural infection as well as to live attenuated vaccines were initiated.

Although it had been recognized that the primary cause of anemia was a secondary consequence of iron deficiency, further studies were conducted in order to look at the question of bioavailability of iron from various sources. Additional emphasis was placed on studies identifying functional abnormalities associated with poor iron nutriture distinct from the functional abnormalities associated with anemia.

The important problem associated with health consequences arising from a different or changing dietary pattern in developing countries was addressed. The importance of identifying changes in incidence of various diseases having an association with a nutritional variable was stressed. The changing dietary patterns in developing countries and the so-called westernization of the diet offers a relatively unique opportunity to conduct such studies in a prospective manner.

Preparation of soyabean cakes for a community feeding center in Thailand.

SUMMARY OF THE RESEARCH ACCOMPLISHMENTS WITHIN THE MAJOR PRIORITY AREAS

Influence of Environmental and Host Factors on Nutritional Requirements

The panel has continued to identify a need for emphasis on the definition of essential nutrient requirements for populations living under conditions of chronic environmental and/or physiological stress. Included within this area is the need to understand apparent physiological adaptations to varying levels of nutrient intake.

Non-invasive stable isotopes of carbon, hydrogen, and nitrogen have been used to investigate adaptive changes in whole-body metabolism of essential amino acids in adults following changes in the intake of dietary protein and energy. When protein intake was varied within the adequate range, the major adaptive change in leucine metabolism resulted from an alteration in leucine oxidation with little change in whole-body protein synthesis and breakdown. At the submaintenance range of protein intake, however, the adaptive response of leucine metabolism to a change in protein intake involved reduced rates of whole-body protein synthesis and breakdown with changes in leucine oxidation contributing a minor role in the economy of leucine metabolism. At the same time, whole-body alanine synthesis appears to increase with the ingestion of energy-containing meals, suggesting a role for alanine in maintaining whole-body nitrogen economy as well as energy storage. In contrast, changes in glycine metabolism do not suggest a major role for this amino acid in maintaining whole-body nitrogen economy, although its rate of synthesis is adaptively reduced when protein intake is limited. Continuation of these studies is expected to be of value in determining the requirements for dietary essential amino acids in human subjects.

In other studies, the influence of dietary protein level, energy sources, and dietary protein energy ratio on the utilization of protein energy has been studied in weanling rats. The level of protein in a diet, the ratio of protein to dietary energy, and the ratio of nonprotein energy from fat versus carbohydrates in the diet influence the rate of weight gain and gross carcass composition. This work has provided information about interactions among dietary concentrations of protein, carbohydrates, and fats. Other studies have shown that pregnant primates can withstand diets containing as little as 3.35% protein (casein) without obvious ill effects to themselves. Although this level of protein intake is well below that of most populations, the offspring were normal when tested by numerous physiological, behavioral, and growth measures, provided the babies were well fed after birth.

Health Significance and Methods of Preventing Iron Deficiency

In previous years, the panel emphasized studies of health significance and methods for preventing nutritional anemia. As a result of this research, iron deficiency surfaced as the primary nutritional precursor of anemia. It also became clear that iron deficiency undoubtedly has other serious manifestations as well. In light of this, the priority area was changed to deal more directly with iron deficiency and its detrimental effects on work capacity, performance, physical/mental development and functions, susceptibility to infections, and perinatal morbidity and mortality. In addition, the panel continues to be interested in developing the means by which iron deficiency can be combatted, including laboratory assessments of iron status, prevalence and cause of iron deficiency, food iron availability, and the efficacy of intervention programs dealing with iron supplementation and iron fortification of food.

Studies of the onset of iron deficiency have shown that hematocrit levels decrease before liver iron falls to its lowest concentration and that cytochrome c in the intestinal mucosal is depressed below control levels even before significant anemia has developed in a rat model. These findings suggest a maximal rate at which iron stores can be mobilized. In the depletion-repletion studies with rats, iron repletion has demonstrated an association with an increase in maximal speed and endurance. These rats were subjected to a sprint-type performance test to establish maximum speed and endurance.

Other studies have shown a decrease of red blood cell survival in iron deficiency states and that the red blood cell membrane under these conditions appeared to have increased membrane stiffness. Decreased red blood cell deformability in iron deficiency has been shown to be due primarily to decreased membrane viscoelasticity. Iron deficient red blood cells have increased susceptibility to peroxidation and have shown evidence for spontaneous membrane lipid and protein cross-linking. This suggests that the increased red blood cell membrane stiffness in iron deficiency may be related to the peroxide damage and may be responsible for the observed decrease in red blood cell survival.

In studies designed to differentiate the effects of anemia from tissue iron deficiency itself, exchange transfusion was employed in rats to control hemoglobin concentration in iron-deficient and control animals. Both brief, hard exercise and prolonged submaximal exercise (endurance) were markedly impaired in the iron-deficient animals. In the exchanged transfused iron deficient rats, aerobic work capacity and maximal oxygen consumption were corrected, but endurance capacity of the iron-deficient exchanged transfused animals was not significantly improved. These results indicate that the defects of maximal oxygen consumption and aerobic work capacity in iron-deficient rats results primarily from anemia and impaired oxygen delivery, whereas the loss of endurance capacity is a direct function of the diminished capacity of muscle mitochondria to utilize oxygen and produce ATP.

Interaction of Nutrition, Immune Competence, and Infection

This difficult but important area remains essentially unchanged. Well-designed, controlled studies are needed to characterize the nutritional status and cell-mediated immunological responses to viral, bacterial, and parasitic functions in defined human populations. In addition, studies are needed on the effect of nutritional alterations on immune competence in animal models and the impact of nutritional intervention programs on the immune status of malnourished human populations.

Studies have demonstrated that the splenic lymphocyte stimulation response to mitogenic doses of phytohemagglutinin in protein-malnourished rats is reduced markedly. Using a crossover type of experiment, results were obtained which suggested that the defect resides in a lymphocyte factor rather than in a serum factor. However, the serum zinc and plasma ascorbic levels were only about half those of control animals, suggesting that a multiple dietary deficiency may have been involved.

Studies investigating the effect of iron status on immunity in neonatal and maternal rats have revealed that individual components of the immune response are affected differently by iron deficiency. While circulating immunoglobulins (IgG or IgM) are resilient to change, the development of lymphoid organs such as the thymus and spleen is profoundly affected by iron deficiency and may alter permanently the immunocompetence of the organism.

Interaction of zinc status and the development of the immune system has also been studied. Using the inbred A/J mouse as a model system, zinc deficiency *in utero* was observed to lead to an interruption in the development of the immune system *in utero*. Suboptimal immune responses persisted after birth despite adequate nutriture as evidenced by normal body weights and serum zinc levels. This observation may explain the persistence of infections and high mortalities observed among low birth-weight infants. Other studies have investigated the marginal deficiencies of zinc and/or copper on *in vivo* response to infection using *Salmonella typhimurium* as the infectious organism. Although mortality rates among the animals within the different dietary groups studied were not different, differences were observed in morbidity indices, hematological responses, spleen weights, and liver weights when animals with sufficient and insufficient intakes were compared.

Finally, a longitudinal study carried out on small-for-dates intrauterine growth-retarded infants and normal weight full-term infants has been initiated in order to determine the effects of intrauterine malnutrition on immune function. At birth, B lymphocytes and immunoglobulins were normal and comparable in both birth groups. Adult levels of IgG, IgM, and IgE were achieved in the intrauterine growth-retarded infants by 7 months of age. Total lymphocyte counts, T cells, and percent T cells were significantly reduced at birth in the intrauterine growth-retarded group. This abnormality persisted at 1 year. Cell-mediated immunity was significantly correlated with birth weight, iron and thiamine at birth, and with folate, hemoglobin, pyridoxine, and riboflavin at 6 and 12 months of age.

Effects of Nutrition on Physical and Mental Development, Behavior, Physical Capability, and Work Performance

The emphasis in the priority area has been on the effects of malnutrition in pregnancy and childhood on virtually all aspects of human function—including lactation, reproductive capabilities, and work performance.

In addition to studies mentioned above, additional observations relating the effects of altered nutritional status on behavioral or performance characteristics have been completed. It was shown that monkeys that were fed low protein diets during the last four fifths of their pregnancy gave birth to infants equivalent in every respect to those born to mothers fed diets rich in protein. Birth weight, subsequent growth rate, and behavioral capacity were entirely comparable. Moreover, the low protein diets seem to have no adverse effects on the mother, except that weight gain during pregnancy was affected. These observations suggest that the pregnant monkey is able to adapt considerably, presumably by improving metabolic efficiency during pregnancy.

A rapid dark adaptation test has been developed which can be used under field conditions on children as young as 4 years of age. This method is a very sensitive test for vitamin A deficiency and is based upon the matching of intensities of colored disc to cone function (not rod function) so that under dim lighting conditions, the ability to separate the different colored disc is dependent on rod function alone. This rapid dark adaptation test is expected to become widely used in surveys to identify subclinical vitamin A deficiency states.

In a longitudinal study of maternal zinc status during lactation it was observed that the calculated dietary intake of apparently healthy, well-nourished, middle-income subjects averaged less than 50% of the RDA. Plasma levels during the first 4 months of lactation remained higher than the mean observed during the third trimester of pregnancy, but below the value found in nonlactating control women of child-bearing age. After 4 months of lactation, plazma zinc levels gradually declined to a low of 65 ± 12 μg/dl at 11 months' lactation. Women who receive zinc supplementation (approximately 13 mg/day) showed a similar pattern in the early months but did not decline with prolonged lactation. Breast milk zinc concentrations declined as lactation progressed; however, the means of the zinc supplemented group were higher at every month of lactation. The observed suboptimal maternal zinc status may be of greater practical importance in determining the zinc intake of older infants and growing children who continue to derive most of their nutrients from breast feeding after 6 months of age.

Health Consequences of Different (and Changing) Dietary Patterns and Food Habits

This area is primarily of concern to the developed countries and those which are undergoing rapid changes in their social, cultural, and physical environments. Selected studies are needed on the long-term consequences of changing dietary patterns, food habits, and the suggested relationship between dietary statistics of health disorders. These include: (a) epidemiological studies on nutritionally linked health disorders related to food habits, (b) studies of the effects on nutrient utilization of food intake patterns, including nutrient intake, balance, and frequency of intake; and (c) a study designed to evaluate the health impact of nutritional interventions in populations.

Unfortunately, some problems of malnutrition remain with us despite the technological advances in food production. In studies of the nutritional value of commonly available foods for infants and children, both ordinary rice and wheat were unable to support normal growth in children when used to provide the only source of protein in the diet. Potatoes could be fed to provide up to 50% of total calories without difficulty. Apparent nitrogen retention was 78% compared to casein control values.

Indonesian baby holding growth chart as part of a prevention program.

which was more than from rice and 50% more than from wheat. It was subsequently found that a diet containing wheat to provide 50% of calories and 80% of the protein and casein to supply 20% of the protein supported excellent growth of children even though plasma amino acid profiles indicated that the amino acid lysine was still somewhat limiting. However, a diet with 75% of the energy and all of the protein coming from wheat seemed unable to support adequate growth in most infants. The addition of lysine to correct the low lysine intake made growth adequate in all respects.

In another study, dietary zinc supplementation in preschool-aged children of Mexican-American ethnic origin from low-income homes significantly increased linear growth velocity in boys, but not in girls. Only the children in the lowest tenth percentile of height for weight were included in the study. These results indicate that low dietary zinc intake is a specific environmental factor which may contribute to poor linear growth velocity in preschool children.

A PROGRESS REPORT ON THE REVIEW OF THE SCIENTIFIC AND ADMINISTRATIVE GOALS OF THE U.S. MALNUTRITION PANEL

In the summer of 1984, a review committee of U.S. scientists familiar with international nutrition was appointed by the NIADDK Director with a charge to identify those areas of greatest scientific opportunity in nutrition which should appropriately be considered by the U.S.-Japan Malnutrition Panel in the next sequence of time. Significant emphasis was placed upon opportunities for collaborative scientific interaction between American and Japanese scientists. The committee delivered its report to the Acting Director of NIADDK in the fall of 1984 and the report was the subject of a special meeting of the U.S. Malnutrition Panel in November 1984. On the basis of those recommendations and the panel meeting, the U.S. panel formulated suggestions for consideration by the Japanese panel for the July 1985 workshop, a part of the ongoing review under the auspices of the U.S.-Japan Committee. Among the new scientific areas identified for potential emphasis by the U.S.-Japan Malnutrition Panel were: osteoporosis and other metabolic forms of bone disease influenced by nutrition; the application of newer technologic developments to the task of nutrition assessment both in the field and in the clinic; a study of the endogenous mediators released in response to infection and an exploration of the mechanisms by which these agents influence nutritional status (e.g., interleukins, leukotrienes, etc.); the nutritional significance of unsaturated fatty acids in fish oils, with particular attention to the prevention of cardiovascular disease; and the biological significance of sulfur amino acids.

The Advisory Committee observed that scientific workshops were an important means of interaction between American and Japanese scientists but should not be considered an end in themselves. In particular, it was recommended that the workshops be used as a basis to foster research interactions to the extent possible. The report also observed a need for some modification of administrative commitments and procedures within NIADDK, which administers the U.S. Malnutrition Panel, if greater goals of scientific interaction and scientific productivity were to be met.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
GRANTS	
V.E. Charlton University of California at San Francisco	Fetal nutrient therapy: Effects on growth retardation
F. Chytil Vanderbilt University	Vitamin A in growth and development
P.R. Dallman University of California at San Francisco	Developmental aspects of iron nutrition
P.J. Fraker Michigan State University	Dietary zinc: Its effect on the immune response
J.H. Freeland-Graves University of Texas at Austin	Saliva: An assessment of Zn nutriture in man
W.Y. Fujimoto University of Washington	Japanese-American community diabetes study (human)
M.E. Gershwin University of California Davis	Zinc deprivation and teratogenesis

Investigator and Institution	*Title*
J.F. Halsey University of Kansas	Effects of colostrum on immune responsiveness
K.M. Hambidge University of Colorado	Studies of human zinc deficiency
M.G. Herrera Harvard University	Malnutrition and child development
B. Lozoff Case Western Reserve University	Iron deficiency anemia and infant behavior
F.H. Morriss, Jr University of Texas	Maternal nutritional regulation of fetal growth
P.M. Newberne Massachusetts Institute of Technology	Influence of trace element nutrition on infection
A.R. Sherman University of Illinois, Urbana-Champaign	Iron nutriture and the development of immunocompetence
P.E. Stake University of Connecticut - Storrs	Bioavailability of supplemental dietary iron
E.R. Stiehm University of California at Los Angeles	Cellular immunity in sick and malnourished infants
W.A. Walker Massachusetts General Hospital	Effect of colostrum on gut maturation and host defense
V.R. Young Massachusetts Institute of Technology	Dietary protein intake and human protein metabolism

BIBLIOGRAPHY

Beach, R.S., M.E. Gershwin, and L.S. Hurley. Altered thymic structure and mitogen responsiveness in postnatally zinc-deprived mice. *Devel. Comp. Immunol.* *3*:725-738, 1979

Beach, R.S., M.E. Gershwin, and L.S. Hurley. Growth and development in postnatally zinc-deprived mice. *J. Nutr.* *110*:201-211, 1980

Beach, R.S., M.E. Gershwin, R.K. Makishima, and L.S. Hurley. Impaired immunologic ontogeny in postnatal zinc deprivation. *J. Nutr.* *110*:805-815, 1980

Beach, R.S., M.E. Gershwin, and L.S. Hurley. Dietary zinc modulation of Moloney sarcoma virus oncogenesis. *Canc. Res* *41*:552-559, 1981

Beach, R.S., M.E. Gershwin, and L.S. Hurley. Nutritional factors and autoimmunity. I. Immunopathology of zinc deprivation in New Zealand mice. *J. Immunol.* *126*:1999-2006, 1981

Beach, R.S., M.E. Gershwin, and L.S. Hurley. Nutritional factors and autoimmunity. II. Prolongation of survival in zinc-deprived NZB/W mice. *J. Immunol.* *128*:308-313, 1982

Beach, R.S., M.E. Gershwin, and L.S. Hurley. Reversibility of developmental retardation following murine fetal zinc deprivation. *J. Nutr.* *112*:1169-1181, 1982

Beach, R.S., M.E. Gershwin, and L.S. Hurley. Nutritional factors and autoimmunity. III. Zinc deprivation versus restricted food intake in MRL/1 mice – The distinction between interacting dietary influences. *J. Immunol.* *129*:2686-2692, 1982

Beach, R.S., M.E. Gershwin, and L.S. Hurley. Gestational zinc deprivation in mice: Persistence of immunodeficiency for three generations. *Science* *218*:469-471, 1982

Beach, R.S., M.E. Gershwin, and L.S. Hurley. Persistent immunological consequences of gestational zinc deprivation. *Ame J. Clin. Nutr.* *38*:579-590, 1983

Blum, P.M., D. Phelps, B.J. Ank, H.J. Krantman, and E.R. Stiehm. Survival of oral human immune serum globulin in the gastrointestinal tract of low birth weight infants. *Pediatric Research* *15*:1256-1260, 1981

Bresson, J.L., W.A. Walker. Comparison of glycoprotein and glycolipid components of newborn and adult intestine microvillus membrane: Use of cholera toxin as a lectin. *Gastroenterology* *80*:1116, 1981

Bresson, J.L., A. Hercovics, and W.A. Walker. Differences in microvillus membrane glycoprotein fucosylation with age and differentiation. *Gastroenterology* *82*:1025, 1982

Carney, J.M., M.S. Warner, T.C. Borut, W. Byrne, M.E. Ament, J.D. Cherry, and E.R. Stiehm. Cell mediated immune defects and infection. A study of malnourished hospitalized children. *Amer. J. Dis. Child.* *134*:824-827, 1980

Charlton, V. and A.M. Rudolph. Digestion and absorption of carbohydrates by the fetal lamb *in utero*. *Pediatr. Res* *13*:1018-1023, 1979

Charlton, V., B. Reis, and D. Lofgren. Consumption, rather than gain, of carbohydrates, amino acids and oxygen across the intestinal circulation in the chronically catheterized fetal lamb *J. Develop. Physiol.* *1*:329-336, 1979

Charlton, V. and B. Reis. Effects of gastric nutritional supplementation on fetal umbilical uptake of nutrients. *Amer. J Physiol.* *241*:E178-185, 1981

Charlton, V. and R. Phibbs. Peripheral venous partial exchange transfusions for neonatal polycythemia. *Clin. Ped.* *22*:5960, 1983

Charlton, V. Fetal nutritional supplementation. *Seminars in Perinatol.* 8:25-30, 1984

Charlton, V. and M. Johengen. Nutrient waste product concentration differences in fetal upper and lower body arteries *J. Develop. Physiol.*, 1984, in press

Charlton, V. Nutrient administration to the fetus via gastrointestinal, vascular and amniotic routes. *In* Animal Models in Fetal Medicine IV. (P. Nathanielsz ed.), Perinatology Press, Ithaca, New York, 1984, in press

Chytil, F. Vitamin A and skin. *In* Biochemistry and Physiology of the Skin (L.A. Goldsmith, ed.) Oxford University Press, pp 1187-1199, 1983

Chytil, F. and D.E. Ong. Retinol-binding proteins and human cancer. *In* Molecular Interrelations of Nutrition and Cancer (M.S. Arnott, J. van Eys, and Y.W. Wang eds.) Raven Press, pp. 409–417, 1982

Chytil, F. and D.E. Ong. Cellular retinol and retinoic acid binding proteins. *In* Advances in Nutritional Research (H.H. Draper ed.) Plenum Press, New York, pp. 13-28, 1983

Chytil, F. Liver and cellular vitamin A binding protein *Hepatology* 2:282-287, 1982.

Chytil, F. *Vitamin A and Skin in Physiology and Biochemistry of the Skin* (L.A. Goldsmith, ed.) Oxford University Press, pp 1187-1199, 1983

Chytil, F., M. Omori, G. Liau, and D.E. Ong. Retinoids, cellular retinoid binding proteins, nucleus and cancer. *In Press*: Diet and Cancer. From Basic Research to Policy Implications. (D. Roe, ed.) A.R. Liss, New York, pp. 117-123, 1983

Chytil, F. and D.E. Ong. Cellular retinoid binding proteins Chapter in *The Retinoids* (D.S. Goodman and M.B. Sporn, eds.) vol. 2, pp. 89-123, Academic Press, New York, 1984

Chytil, F. Retinoids and Large Bowel Cancer. Chapter in *Progress in Cancer Research and Therapy* (S.R. Wolman and A.J. Mastromarino) Raven Press, New York, pp. 361–365, 1984.

Chytil, F. Retinoids, intracellular binding proteins, and mechanism of action in Retinoid Symposium, Geneva, 1984.

Chytil, F. Retinoic Acid: Biochemistry, Pharmacology, Toxicology and Therapeutic Use. *Pharmacol. Reviews 36*:935–1005, 1984.

Dallman, P.R., M.A. Siimes, and A. Stekel. Iron deficiency in infancy and childhood. *Am. J. Clin. Nutr. 33*:86–118, 1980.

Dallman, P.R. Inhibition of iron absorption by certain foods. *Am. J. Dis. Child. 134*:453–454, 1980.

Dallman, P.R. Serum ferritin in the diagnosis of iron deficiency. *In: Radioimmunoassay of Hormones, Proteins and Enzymes*. A. Albertini, ed. Excerpta Medica, Amsterdam, 1980, pp. 230–238.

Dallman, P.R. Anemia of prematurity. *Ann. Rev. Med 32*:143–160, 1981.

Dallman, P.R. Iron deficiency — diagnosis and treatment. *West J. Med. 134*:496–505, 1981.

Dallman, P.R., J.D. Reeves, D.A. Driggers, and E.Y.T. Lo. Diagnosis of iron deficiency: the limitations of laboratory tests in predicting response to iron treatment in one-year-old infants. *J. Pediatr.* 99:376–381, 1981.

Dallman, P.R. Iron nutrition of infancy in *Infant and Child Feeding*, J.T. Bond, L.J. Filer, G.A. Leveille, A. Thompson, and W.W. Weil, Eds., Academic Press, New York, 1981; pp 363–376.

Dallman, P.R. Diagnostic Criteria for Iron Deficiency in *Iron Nutrition Revisited-Infancy, Childhood, Adolescence*, Ross Conference, 1981, pp. 3–15.

Dallman, P.R. Nutritional anemias: relationship to genetic and pharmacologic factors. In *Nutrition in the 1980's Constraints on our Knowledge*. Alan R. Liss, Inc. Proceedings of the West Hemisphere Nutr. Congress 1980, Los Angeles, pp. 87–96, 1981.

Dallman, P.R. Iron deficiency and related nutritional anemias in *Hematology of Infancy and Childhood*, 2nd edition. D. Nathan and F.A. Oski, eds. W.B. Saunders, Philadelphia, 1981, pp. 298–343.

Dallman, P.R. Manifestations of iron deficiency. *Sem. Hematol 19*:19–30, 1982.

Dallman, P.R., C.A. Refino, and M.J. Yland. Sequence of development of iron deficiency in the rat. *Am. J. Clin. Nutr 35*:671–677, 1982.

Dallman, P.R. Iron deficiency: distinguishing the effects of anemia from muscle iron deficiency on work performance in *Proceedings of the 5th International Conference* on Proteins of Iron Storage and Transport, La Jolla, 1981. Elsevier, New York, 1982, pp. 509–524.

Dallman, P.R. Biochemical and hematologic indices of iron deficiency in *Iron Deficiency, Brain Biochemistry and Behavior*, E. Pollitt and R. Leibel, eds. Raven Press, New York, 1982, pp. 63–77.

Dallman, P.R., R. Yip, and C. Johnson. Methods for estimating the prevalence of iron deficiency and iron deficiency anemia in industrialized countries in *Groups With High Risk of Iron Deficiency in Industrialized Countries*. D. Dupin and S. Hercberg, eds., INSERM, Paris, 1983, pp. 21–31.

Dallman, P.R., R. Yip, and C. Johnson. Prevalence and causes of anemia in the United States, 1976–1980. *Am. J. Clin. Nutr. 39*:437–445, 1984.

Dallman, P.R. and J.D. Reeves. Laboratory diagnosis of iron deficiency in *Iron Nutrition in Infancy and Childhood*. A. Stekel, ed. Raven Press, New York, 1984, pp. 11–44.

Dallman, P.R. Diagnosis of anemia and iron deficiency: analytic and biologic variations of laboratory tests. *Am. J. Clin. Nutr. 39*:937–941, 1984.

Dallman, P.R. Iron Deficiency in Current Therapy. H.F. Conn, et al., eds. Saunders, Philadelphia, 1983, pp. 245–249.

Erickson, K.L., C.J. McNeill, and M.E. Gershwin. Influence of dietary fat concentration and saturation on immune ontogeny in mice. *J. Nutr. 110*:1555–1572, 1980.

Gershwin, M.E., D.R. Lentz, R.S. Beach, and L.S. Hurley. Nutritional factors and autoimmunity IV. Dietary vitamin A deprivation induces a selective increase in IgM autoantibodies and hypergammaglobulinemia in New Zealand black mice. *J. Immunol. 133*:222, 1984.

Golub, M.S., M.E. Gershwin, L.S. Hurley, A.G. Hendrickx, and D.L. Baly. Induction of Marginal Zinc Deficiency in Female Rhesus Monkeys. *Amer. J. Primatol. 3*:299–305, 1982.

Golub, M.S., M.E. Gershwin, and V.K. Vijayan. Passive avoidance performance of mice fed marginally or severely zinc deficient diets during post-embryonic brain development. *Physiol. Behav. 30*:409–413, 1983.

Golub, M.S., M.E. Gershwin, L.S. Hurley, D.L. Baly, and A.G. Hendrickx. Studies of marginal zinc deprivation in rhesus monkeys. I. Influence on pregnant dams. *Amer. J. Clin. Nutr 39*:265–280, 1984.

Golub, M.S., M.E. Gershwin, L.S. Hurley, D.L. Baly, and A.G. Hendrickx. Studies of marginal zinc deprivation in rhesus monkeys: II. Pregnancy outcome. *Amer. J. Clin. Nutr 39*:879–887, 1984.

Hambidge, K.M. Chromium. *In:* Disorders of Mineral Metabolism, Volume 1. (F. Bronner and J.W. Coburn, eds.). Academic Press, New York, 1981, pp. 271–289.

Hambidge, K.M. The need for trace element analyses in the medical sciences. *In*: Technical Report on Elemental Analysis of Biological Materials. International Atomic Energy Agency, Vienna, 1980, pp. 19–28.

Hambidge, K.M. and C.E. Casey. Trace element requirements in premature infants. *In*: Textbook of Gastroenterology and Nutrition in Infancy, (E. Lebenthal, ed.), Raven Press, New York, 1981, pp. 595–610.

Hambidge, K.M. Zinc deficiency in man: Its origins and effects. *In*: Philosophical Transactions of the Royal Society, London, B294:129–144, 1981.

Hambidge, K.M. and P.A. Walravens. Disorders of Mineral Metabolism. *In*: Clinics of Gastroenterology, (J.T. Harries, ed.), WB Saunders Company, Ltd., London, Volume 11, Number 1, 1982, pp. 87–118.

Hambidge, K.M. Zinc in human nutrition. *In*: The Proceedings of the Florida Symposium on Micronutrients in Human Nutrition, (P.A. Wagner and J.R. Kirk, eds.), Institute of Food and Agricultural Sciences, 1981, pp. 35–46.

Herrera, M.G., J.O. Mora, N. Christiansen, N. Ortiz, J. Clement, L. Vuori, B. Paredes, M. Wagner, and D. Waber. Effects of nutritional supplementation and early education on physical and cognitive development. *In: Life-Span Developmental Psychology, Intervention*, (R.R. Turner and F. Reese, eds.) Academic Press, Inc., New York, 1980, pp. 149–184.

Herrera, M.G., J.O. Mora, B. Paredes, and M. Wagner. Maternal weight/height and the effect of food supplementation during pregnancy and lactation. *In: Maternal Nutrition during Pregnancy and Lactation*, (H. Abei and R. Whitehead, eds.) Huber, Vienna, 1980, pp. 252–263.

Kleinman, R.E., P.R. Harmatz, and W.A. Walker. The liver: An integral part of the enteric mucosal immune system. *Hepatology 2*:379–384, 1982.

Kochanowski, B.A. and A.R. Sherman. Decreased antibody formation in iron-deficient rat pups—effect of iron repletion. *Amer. J. Clin. Nutr.* (in press).

Lozoff, B., G.M. Brittenham, F.E. Viteri, and J.J. Urrutia. Growth and development in iron deficiency anemia. Presented in Boston, 1981. *Society for Research in Child Development Abstracts 3*:174, 1981.

Lozoff, B., G.M. Brittenham, J.J. Urrutia, and F.E. Viteri. Behavior and development of iron deficient anemic infants. *Ped Res. 15*:588, 1981.

Lozoff, B., G.M. Brittenham, F.E. Viteri, and J.J. Urrutia. Behavioral abnormalities in infants with iron deficiency anemia. *In: Iron Deficiency: Brain Biochemistry and Behavior*. E. Pollitt and R. Leibel, eds., Raven Press, New York, pp. 183–194, 1982.

Lozoff, B., G.M. Brittenham, F.E. Viteri, A.W. Wolf, and J.J. Urrutia. The effects of short-term oral iron therapy on developmental deficits in iron deficient anemic infants. *J. Pediatr. 100*:351–358, 1982.

Lozoff, B., G.M. Brittenham, F.E. Viteri, A.W. Wolf, and J.J. Urrutia. Developmental deficits in iron-deficient infants: Effects of age and severity of iron lack. *J. Pediatr. 101*:948–952, 1982.

Lozoff, B. Reply to L. Avramidis. Developmental deficits in iron-deficient children. *J. Pediatr. 103*:339–340, 1983.

Lozoff, B. and A. Wolf. Does abnormal behavior account for low Bayley scores in iron deficient infants? Presented at American Pediatric Society, 1983, Washington, D.C. *Ped. Res. 17*:100A, 1983.

Lozoff, B., A.W. Wolf, J.J. Urrutia, and F.E. Viteri. Abnormal behavior and low developmental test scores in iron-deficient anemic infants, 1984. *J. Dev. Beh. Peds.*, in press.

Mora, J.O., M.G. Herrera, S.G. Sellers, and N. Ortiz. Nutrition, social environment and cognitive performance of disadvantaged Colombian children at three years. *In: Nutrition in Health and Disease and International Development: Symposia from the XII International Congress of Nutrition*. Alan R. Liss, Inc., New York, 1981, pp. 403–420.

Mora, J.O., M.G. Herrera, and J. Suescun. The effect of food supplementation on the physical growth of undernourished preschool children. 1984, in preparation.

Mora, J.O., M.G. Herrera, J. Suescun, L. Navarro, and M. Wagner. The effects of nutritional supplementation on physical growth of children at risk of malnutrition. *Am. J. Clin. Nutr. 34*:1885–1892, 1981.

Mora, J.O., M.G. Herrera, J. Suescun, and B. Paredes. Timing of supplementary feeding and its effects on physical growth of disadvantaged children. 1984, in preparation.

Mora, J.O., R. Sanchez, B. Paredes, and M.G. Herrera. Sex-related effects of nutritional supplementation during pregnancy on fetal growth. *Early Human Development 5*:243–251, 1981.

Mora, J.O., S.G. Sellers, J. Suescun, and M.G. Herrera. The impact of supplementary feeding and home education on physical growth of disadvantaged children. *Nutrition Research 1*:213–225, 1981.

Morriss, F.H., C.R. Rosenfeld, S.S. Crandell, and E.W. Adcock. Effects of fasting on uterine blood flow and substrate uptake in sheep. *J. Nutr. 110*:2433–2443, 1980.

Morriss, F.H. Placental factors conditioning fetal nutrition and growth. *In*: J. Metcoff (ed.): Nutrition of the Child: Maternal Nutritional Status in Fetal Outcome. *Am. J. Clin. Nutr., (Supplement) 34*:760–768, 1981.

Morriss, F.H. and R.M. Caprioli. Techniques and investigation of trace element metabolism in the mother and fetus. *In*: P.E. Nathanielsz (ed.): *Animal Models in Fetal Medicine, vol. II*, Elsevier, Amsterdam, 1982, pp. 150–190.

Morriss, F.H., L.M. Lichtenberger, S.S. Crandell, and P.A Palma: Contraplacental hypogastrinemic effect of gastrin infusion in sheep. *Pediatr. Res. 18*:528–531, 1984

Morriss, F.H. Infants of diabetic mothers: Fetal and neonatal pathophysiology. *Perspect. Pediatr. Pathol. 8*:223–234, 1984.

Morriss, F.H., B. Fitzgerald, and L. Riddle. Decreased uptake of sulfate S04 by cartilage of fetal sheep perfused with S04 compared to their twins. *Pediatr. Res. 18*:142A, 1984

Nauss, K.M., A.M. Connor, A. Kavanaugh, and P.M Newberne. Alterations in immune function in rats caused by dietary lipotrope deficiency: effect of age. *J. Nutr 112*:2333–2341, 1982

Nauss, K.M., A.M. Connor, and P.M. Newberne. Alterations in immune function in rats caused by dietary lipotrope deficiency: Effect of culture medium, 2-mercaptoethanol and mitogen dose on the *in vitro* lymphocyte transformation response. *J Nutr. 112*:2342–2352, 1982.

Nauss, K.M. and P.M. Newberne. Effects of dietary folate, vitamin B_{12} and methionine choline deficiency on immune function. *In: Diet and Resistance to Disease*: Plenum Publishing Co., New York, N.Y., 63-91, 1981

Nauss, K.M. and P.M. Newberne. Trace elements and immunocompetence. *In: Trace Element Metabolism in Man and Animals*: Proceedings of TEMA-4, Perth, Western Australia May 11–15, 1981, Australian Academy of Science, Canberra, 603–612, 1981.

Newberne, P.M. Disease states and tissue mineral elements in man. *Federation Proceedings Vol. 40*: No. 8, June 1981

Newberne, P.M. Dietary fat, immunological response and cancer in rats. *Cancer Res. 41*:3783–3785, 1981.

Palma, P.A., S.S. Crandell, and F.H. Morriss. Lack of detectable change in ovine umbilical uptake of individual amino acids during uterine artery insulin perfusion. *Pediatr. Res. 14*:459, 1980

Rothenbacher, H. and A.R. Sherman. Target organ pathology in iron-deficient suckling rats. *J. Nutr. 110*:1648–1654, 1980

Rothman, D., M.C. Lathman, W.A. Walker. Transport of macromolecules in malnourished animals. I. Evidence for increased uptake of intestinal antigens. *Nutrition Research 3*:467–473, 1982.

Sherman, A.R. Immunoglobulins and lysozyme in iron-deficient and iron-overloaded rats. *International 29*:859–868, 1984

Sherman, A.R., B.A. Kochanowski, and I. Wolinsky Lysozyme and other measures of immunity in young, mature, and aged rats. *Gerontology 30*:120–124, 1984

Sherman, A.R. Liver and adipose tissue enzymes in iron-deficient rat pups and dams. *Baroda J. Nutr. 10*:47–53, 1983.

Sherman, A.R. Iron, infection and immunity. *In*: "Malnutrition, Disease Resistance, and Immune Function." (R.E Watson, ed.), Marcel Dekker Publisher, 1984, pp. 251–266.

Stiehm, E.R., L.H. Kronenberg, H.M. Rosenblatt, Y.J Bryson, and T.C. Merigan: Interferon: Immunobiology and clinical significance. *Ann. Int. Med. 96*:80–93, 1982.

Stiehm, E.R. Use of human immune serum globulins. *Pediatrics in Review 4*:135–143, 1982

Stiehm, E.R., M.B. Sztein, P.S. Steeg, D. Mann, C. Newland, R.M. Blaese, and J.J. Oppenheim. Deficient DR antigen expression on human cord blood monocytes: Reversal with lymphokines. *Clin. Immunol. Immunopath. 30*:430–436, 1984

Stiehm, E.R. Clinical and experimental use of human immune serum globulins in infants and children. *J. Immunopharm. Immunol. 4*:99-107, 1984

Stiehm, E.R. Purine-related enzyme deficiencies and immunodeficiencies. *J. Immunol. Immunopharm. 4*:120-122 1984

Stiehm, E.R., D. Mann, C. Newland, M.B. Sztein, P.S. Steeg, J.J. Oppenheim, and R.M. Blaese. Deficient DR antigen expression on human neonatal monocytes: Reversal with lymphokines in, *Primary Immunodeficiency Diseases* (R.J Wedgwood, F.S. Rosen, N.W. Paul, and Alan R. Liss, eds.) New York, 1983, pp. 295-298

Yang, R.D., D.E. Matthews, D.M. Bier, C. Lo, and V.R Young. Alanine kinetics in man: Influence of different isotopic tracers. *Am. J. Physiol.*, 1984 (in press)

Yang, R.D., L.L. Moldawer, A. Sakamoto, R.A. Kennan, D.E Matthews, R.R. Wolfe, V.R. Young, R.W. Wannemacher, G.L. Blackburn, and B.R. Bistrian. Leukocyte endogenous mediator alters protein dynamics in rats. *Metabolism 32*:654–660, 1983

Young, V.R., M. Gersovitz, and H.N. Munro. Human aging: Protein and amino acid metabolism and implications for protein and amino acid requirements. *In: Nutritional Approaches to Aging Research*. (G.B. Moment, ed.), CRC Press, Boca Raton, FL, Chapter 4, pp. 47-81, 1982

Young, V.R. Protein-energy interrelationships in the newborn: a brief consideration of some basic aspects *In: Gastrointestinal Development and Infant Nutrition* (Emmanuel Lebanthal, ed.), Raven Press, New York, 1981

Young, V.R., N.S. Scrimshaw, and D.M. Bier. Whole body protein and amino acid metabolism: relation to protein quality evaluation in human nutrition. *J. Agri. and Food Chem 29*:440–447, 1981

Young, V.R. and D.M. Bier. Stable isotopes (13C and 15N) in the study of human protein and amino acid metabolism and requirements. *In: Nutritional Factors Modulating Metabolism*. (R. de Beers, ed.), Vol. 13, Chapter 25, Raven Press, New York, pp. 267–308, 1981.

Young, V.R., J.J. Robert, K.J. Motil, D. Matthews, and D.M. Bier. Protein and energy intakes in relation to protein turnover in man. *In: Nitrogen Metabolism in Man* (J.C. Walterlow and J.M.L. Stephen, eds.). Applied Science Publishers, London, Chapter 35, pp. 419-447, 1981.

Young, V.R. Dynamics of human whole body amino acid metabolism: Use of stable isotope probes and relevance to nutritional requirements. *J. Nutr. Sci. Vitaminology* 27:395-413, 1981.

Young, V.R., K.J. Motil, and J.F. Burke. Energy and protein metabolism in relation to requirements in the burned pediatric patient. *In: Pediatric Nutrition.* (R.M. Suskind, ed.) Raven Press, New York, 1981.

Young, V.R. Stable isotopes in nutrition research: Introduction. *Fed. Proc.* 41:2677-2678, 1982.

Young, V.R., R.D. Yang, C. Meredith, D.E. Matthews, and D.M. Bier. Modulation to amino acid metabolism by protein and energy intakes. *In: Amino Acids Metabolism and Medical Applications* (G.L. Blackburn, J.P. Grant, and V.R. Young, eds.). John Wright PSG, Inc., Littleton, Massachusetts, Chapter 2, pp. 13-28, 1983.

Young, V.R. and J.F. Burke. Measuring compartments as an index of protein and amino acid status. *Trauma and Animal Models.* 1984 (in press).

Young, V.R., L.L. Moldawer, R. Hoerr, and D.M. Bier. Mechanisms of adaptation to protein malnutrition. *In: Nutritional Adaptation in Man.* (K.L. Blaxter and J.C. Waterlow, eds.). Applied Science Publishers, London, 1984 (in press).

Joint Scientific Conferences

MALNUTRITION PANELS

Date: August 29-31, 1980

Place: Morioka, Japan

Summary: A workshop entitled "Effects of Nutrition on Human Growth and Development and Later Life and Health: Global Priority Issues" was held August 29-31, 1980, in Morioka, Japan. Discussions focused on the relationship of nutritional status during the fetal, prenatal, and postnatal periods of development and long-term effects over the life cycle. As an outgrowth of this workshop, areas of research needs to better understand the effects of nutrition on human growth and development and adult health were identified. The proceedings of the workshop were published as a monograph by the Japanese Panel on Malnutrition and edited by Goro Inoue and Hiroshi Wako.

Date: August 15-16, 1981

Place: San Diego, California

Summary: During August 15-16, 1981, a workshop was conducted on "Opportunities for Collaborative Research Relating to Nutritional Requirements in Asian Countries" in San Diego, California. This workshop allowed interaction of nutritional scientists from Bangladesh, Burma, China, India, Malaysia, New Guinea, Thailand, Great Britain, Japan, and the United States.

Date: December 7-8, 1982

Place: Tokyo, Japan

Summary: On December 7-8, 1982, a workshop was held in Tokyo, Japan on "Practical Approaches to Eradication of Subclinical Iron Deficiency." In addition to discussions on the evaluation of the status of iron nutriture and the common subsequent development of anemia, the fact that iron deficiency can have widespread functional effects on performance was emphasized. The proceedings were published by the Japanese Panel on Malnutrition as a monograph edited by G. Inoue and R. Suzue.

Date: February 28-29, 1984

Place: Bethesda, Maryland

Summary: A workshop was held February 28-29, 1984, in Bethesda, Maryland entitled "Vitamin A and Cancer Prevention." This workshop was cosponsored by the U.S.-Japan Malnutrition Panels in association with the National Cancer Institute, National Institutes of Health. The meeting focused on epidemiologic and clinical studies related to vitamin A and its possible role in cancer risk. The proceedings of the workshop were published as a supplement to the *Journal of the National Cancer Institute* (Volume 73, pp. 1371-1489, 1984), which was edited by Barbara A. Underwood.

Date: December 6-7, 1984

Place: Tokyo, Japan

Summary: On December 6-7, 1984, a workshop was held in Tokyo, Japan entitled "Calcium Metabolism and Aging." The workshop highlighted areas of active investigation by both United States and Japanese scientists on such topics as the influence of vitamins, age, and environmental factors on calcium metabolism. Several presentations concerning calcium and other nutritional factors relating to osteoporosis were given. The proceedings of the workshop are expected to be published as a monograph.

PARASITIC DISEASES PANEL

[illegible]

PANEL MEMBERS

[illegible]

Dr. Kazuo Yasuraoka (1979–)
Professor
Department of Parasitology
Institute of Basic Medical Science
Tsukuba University
Sakura-mura, Niihari-gun
Ibaraki Prefecture 305, Japan

Former Members

Dr. Yoshito Wada (1975–1980)
Director
Department of Medical Entomology
National Institute of Health
2-10-35, Kamiosaki, Shinagawa-ku
Tokyo 141, Japan

Dr. Muneo Yokogawa, Chairman (1971–1979;
Member 1965–1971)
Professor
Department of Physiology
Faculty of Medicine
Chiba University
1-8-1, Inohana
Chiba-shi 280, Japan

Former Member

Dr. Alfred W. Senft (1976–1980)
Professor of Medical Science
Brown University
26 Halsey Street
Providence, Rhode Island 02906

Japanese Parasitic Diseases Panel

BROAD GOALS

Following the guidelines established in 1965, schistosomiasis and filariasis were the foci of the Joint Panels on Parasitic Diseases. In 1972, the panel reviewers, appointed by the Joint Committee, suggested a possible revision of the guidelines on the basis of progress achieved in the program. Both the Japanese and the U.S. panels have continued the careful review of their research activities and discussed the guidelines at each joint meeting. It was agreed to continue to focus intensive efforts on filariasis and schistosomiasis rather than to distribute efforts thinly on multiple areas. The Japanese panel continued to support research on defined subjects on these two diseases in the following areas:

Pathophysiology
Biochemistry and biology
Chemotherapy and pharmacology
Immunology and immunodiagnosis
Host-parasite relationship
Vector ecology
Epidemiology and control

It was fully realized that for the successful control of filariasis and schistosomiasis in Japan, basic scientific research was crucially important. In this regard, the Japanese panel appreciated the close communication and exchange of information maintained among Japan and U.S. researchers that occurred outside of programmed research activities.

Although successful control of filariasis and schistosomiasis in Japan is encouraging and shows the possibility of eradication of the diseases elsewhere in the world, it should be emphasized that continuous attention must be paid to monitoring the recurrence of epidemics in the formerly infected areas. Actually, in 1970 the Tone River basin experienced an unexpected small epidemic of schistosomiasis in dairy cows pastured along the river bank. Furthermore, in Asia and in the Pacific, either or both of these two diseases are still serious problems in many more areas. Situations in endemic areas obviously vary greatly from area to area and are much more complicated than in Japan. A control measure which had been proven to be successful in one area may not always be effective in, or applicable to, other areas. More sophisticated control measures with multidisciplinary approaches will be necessary. Therefore, comprehensive understanding of the diseases is required, including the basic knowledge acquired by biomedical research in the laboratory. Ecology and biology of vectors, as well as the epidemiology of diseases in the endemic area, must be more thoroughly investigated.

The joint panels who met at Kumamoto in 1982 discussed the Communique of the 18th Joint Committee Meeting in recommending a symposium on the current status of parasitic infections in 1983. The symposium took place in Bethesda, Maryland in July 1983. At that time, the panels drafted revised guidelines to expand the interest to research on other selected helminthic and protozoal diseases. The proposed guidelines were submitted to the Joint Committee for consideration.

PROGRESS IN PRIORITY AREAS

Schistosomiasis

Epidemiology

Epidemiological studies in a village with 1,300 inhabitants in Kenya confirmed that the degree of human contact with water was closely related to the age-sex distributions of prevalence rate and intensity.

In a village in Leyte, Philippines, the annual incidence among primary schoolchildren varied from 22.2% to 28.4% from 1975 to 1979. The incidence was reduced abruptly to 8.4% and 6.8% after the school year 1980–81 by mass chemotherapy with praziquantel.

Two laboratories are engaged in research on the periodicity of cercarial emergence. In outdoor conditions, the peak of emergence of *S. japonicum* cercariae from *O. quadrasi* was between 1800 and 2000 hours, but a second peak appeared between 0800 and 1000 hours. In the laboratory, the peak was primarily between 1600 and 1800 hours without a conspicuous second peak in the morning.

In another investigation, two patterns of emergence of *S. japonicum* cercariae were observed; the initial nonperiodic emergence took place immediately after submerging the snails in water and was followed by periodic diurnal emergence with a peak in the afternoon.

Immunity

One investigator is attempting to determine the role of IgE antibody in protective immunity to schistosomiasis japonica. Adherence of eosinophils and eosinophil-mediated damage to dinitrophenylated mechanical schistosomula (DNP-somula) occurred after treating the somula with anti-DNP IgE antibody but not with normal mouse serum. Enhancement of the adherence and parasite damage was observed when eosinophils from *Nippostrongylus brasiliensis*-infected rats were used. Results of a rosette forming assay carried out by using DNP-sheep erythrocytes suggested that this enhancement may be due to the increased expression of Fc receptors for IgE on eosinophils.

A study has been initiated recently on antigen-specific suppressor T cells (Leu 2a$^+$ 3a$^-$) in low responders to schistosomal worm antigen in humans. Low responders to schistosomal antigen, determined by antigen-specific T cell proliferation *in vitro*, had a significant association with HLA-Bw52-Dw12-DR2. Low responsiveness was controlled by antigen-specific suppressor T cells which were Leu 2a positive. Restriction mapping of the HLA-class II genes using 9 enzymes indicated 5 HLA-class II genes composed of DR β, DC β and new unknown class II genes. These observations suggested that there are at least 3 distinct loci for the HLA-class II molecule.

In one laboratory, attention has been focused on examining sequential changes of the oogram after treatment with praziquantel. When the drug was given orally, the oogram on the next day was characterized by an abrupt reduction of mature eggs and an increase of empty egg shells. In balanced salt solution with praziquantel, miracidia were activated within a few minutes and released from the eggs. It appears that immediately after administration of praziquantel most of the mature eggs ruptured in the host tissues, and this results in the sudden release of a large amount of antigenic substance in the blood circulation.

Filariasis

Epidemiology

Filariasis control campaigns in Okinawa and Kagoshima since the early 1960s were followed up epidemiologically. These studies revealed that endemic disease was successfully controlled by selective DEC treatment. The application of skin tests was considered quite useful as an epidemiological index. Endemic onchocerciasis in San Vicente Pacaya, Guatemala was analyzed epidemiologically during the period 1977-81. The detailed epidemiological features, including prevalence and incidence, were elucidated in this country.

Immunology

In the diagnosis of onchocerciasis and malayan filariasis, ELISA was successfully applied by various workers. In onchocerciasis, the increase of immune complex, reduction of anti-tetanus antibodies, and reduction of reactivity against PPD were demonstrated. In various filariasis infection models, the appearance and duration of specific immunoglobulins were examined. In a rat model, it was found that IgE did not play an important role in the protective immunity against challenge infection. An allergen was purified from *Dirofilaria immitis* which stimulated specific IgE antibodies in the dog and mouse. This substance was present in the excretory and secretory fluids of adults, but not in microfilariae. In a *Brugia*-mouse model, it was found that irradiated L_3 induced a high protective immunity which was transferable by spleen cells from sensitized mice. A sero-epidemiological study showed the presence of a close statistical relationship between adult T cell leukemia and filariasis at all infested areas in Nagasaki.

Patho-physiology

The enzyme activities and chemical nature of globin protease, acetyl-choline esterase, acid phosphatase, and Hb hydrolase were investigated in several filariae. A histological study clarified the involvement of the type I hypersensitivity mechanism in killing onchocercal microfilariae after DEC administration. A biochemical study on the metabolism of developing *B. pahangi* revealed a remarkable change in metabolism following the parasite's shift from insect to vertebrate hosts. In the laboratory maintenance of *L. carinii*, techniques were established to collect the great majority of microfilariae from blood and L_3 from mites. A systematic examination of onchocercal nodules in Guatemala clarified histological features and described parasitic involvement in the Western Hemisphere.

Chemotherapy

A method to test the filaricidal action of various drugs such as DEC was intensively investigated in *Brugia*-jird model. Intrapleural injection of DEC yielded successful results in this study. In jirds, rapid excretion of DEC into urine was observed. A sophisticated device was introduced to examine the macrofilaricidal action of some reagents when administered with DEC in the jird model.

Morphology

Chromosome numbers of the following filariae in the spermatogonial and oogonial cells were clarified as follows: *D. immitis* (n=5), *S. digitata* (n=6, 2n=12 in female and 2n=11 in male), *B. pahangi* (n=5) and *B. malayi* (n=5). A technique was established to count the number of nuclei in CS-NR region of microfilariae. The number was considered to be specific for various filarial species. Ultrastructural observations of adult filariae by SEM revealed that the shape of caudal papillae was useful in the differentiation of filarial species. In mice inoculated subcutaneously with the microfilariae of *D. immitis*, the recovery rate was rather low; this experiment suggested the possibility of using this as a model for onchocerciasis. In the *B. pahangi*-GN hamster model, a method of the intra-testicular inoculation with L_3 led to the conclusion that this model was useful for the evaluation of filaricides.

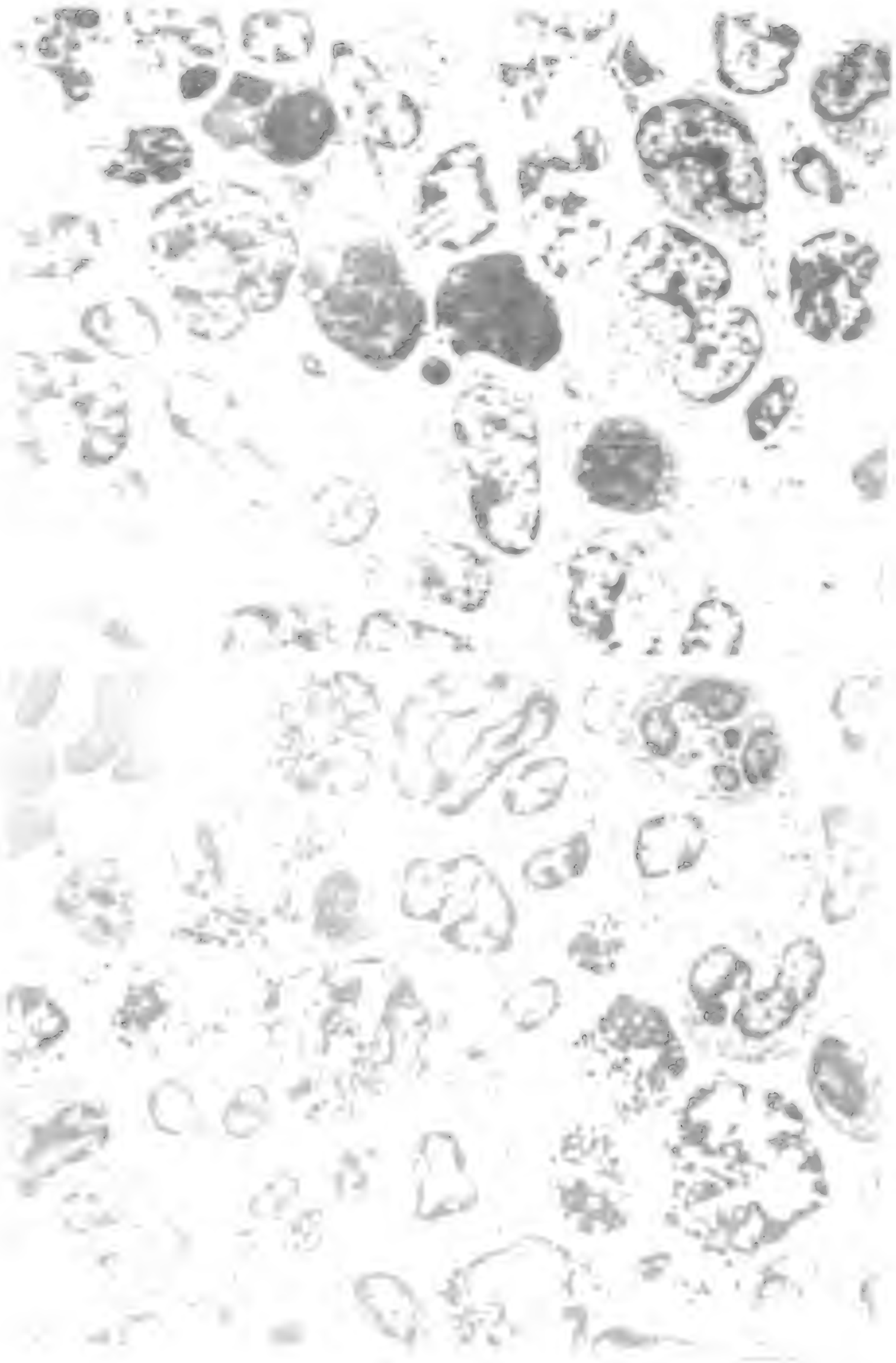
Vectors

The susceptibilities to *B. malayi* of various strains of *Ae. aegypti* and *Ae. togoi* were examined. Two of four strains in the former species and the Taiwan strain in the latter were found to be highly susceptible. By using a *B. malayi*-jird model, the relationships between the blood volume, the number of microfilariae ingested, and the development of larvae were examined. The relationship between microfilarial density and mosquito age was shown to effect the rate of growth of larvae. To study the susceptibility of mosquitoes to filaria, the encapsulation mechanism of microfilariae in the mosquito was investigated. It revealed that encapsulation was related to the nature of the larvae inoculated. A genetic analysis was performed on several species of anopheline mosquitoes from East Asia in regard to their susceptibility to filarial parasites. Methoprene and diflubenzuron, insect growth regulators, were evaluated in the control of blackfly larvae under field conditions. A notable inhibition of adult emergence was caused by the former reagent and a marked decrease of pupal density by the latter. A possible use of these reagents in the control of blackflies was suggested. The transmission dynamics of *D. immitis* by the vector *C. p. pallens* was studied in Nagasaki. In this study, a two-stage catalytic model was applied and was well fitted to the explanation of transmission.

SUMMARY OF ACHIEVEMENTS OF THE PARASITIC DISEASES PANEL DURING THESE 20 YEARS

Since 1965, both basic and applied research on schistosomiasis and filariasis were encouraged and supported by the Japanese panel in accordance with its goals. Epidemiological surveys for schistosomiasis and filariasis were conducted in Japan and some foreign countries. The control of both filariasis and schistosomiasis in Japan was achieved during the 20 years of the U.S.-Japan Program.

No new cases of human schistosomiasis have been found in Japan since the last case at Kofu in 1977. In Japan, control measures were exclusively directed to the elimination of vector



snails. The irrigation ditches in rice fields were cemented, and snails were killed by molluscicides together with reclamation of wet areas and river water management. The intermediate host snail, *Oncomelania nosophora*, was eradicated by 1981 except for the Kofu area where a few snails remain in some isolated sites.

Epidemiological studies on schistosomiasis were conducted at Taveta, Kenya; Bouar, Central Africa; Recife, Brazil; and Leyte, Philippines. The annual incidence observed in the Philippines was not affected much by small scale drainage work but was reduced significantly (from 28.4% to 6.8%) by mass chemotherapy with praziquantel. In the Philippines, the snail *O. quadrasi* was found to live only on the banks of bodies of water and not in the water itself. The snail distribution on the soil is uneven following the double Poisson type. Survey data were successfully normalized by a simple transformation formula log (x + 0.01) which worked better than other more sophisticated ones.

A new promising synthetic molluscicide, *phebrol*, was developed and studied for its characteristics. Among molluscicides of plant origin, extract of *Jatropha curcas* seed is worthy of further study.

In the study on the periodicity of cercarial shedding of *S. haematobium*, shedding was inhibited by darkening the part of the umbilicus of *Bulinus globosus*.

In experimental initiation of liver cancers by 2-FAA in ddY mice, tumors developed with a higher incidence and were more advanced in mice with a preceding infection with *Schistosoma japonicum* than in uninfected ones.

Schistosomula of *S. japonicum* and *S. mansoni* were successfully cultured *in vitro* and developed into adults which showed mating but no oviposition. For killing of the cultured schistosomula, roles of monoclonal antibodies of IgG1, IgG2a and IgE, neutrophils, eosinophils, and complement were clarified. Miracidia of *S. japonicum* were cultured in a balanced salt solution supplemented with other necessary nutrients. The same medium was applicable to the *in vitro* culture of mid-gut tissues of *O. hupensis*. The transplantation of daughter sporocysts of *S. mansoni* was successful in *B. glabrata*. Cercarial shedding was observed in about half of snails in the first and succeeding transplantations.

In human populations, low responsiveness to schistosomal worm antigen was related specifically to T cell proliferation *in vitro*, and had a statistically significant association with HLA-Bw52, Dw12, DR2. Low responsiveness was controlled by the specific suppressor T cells positive for Leu 2 and negative for Leu 3a. Restriction mapping of the HLA-class II genes indicated presence of associating 5 genes composed of DRβ, DCβ and unknown new ones, and suggested that there were at least 3 loci for the HLA-class II molecule. In connection with genetic regulation of immune responses, IgE antibody formation against *S. japonicum* compared in various strains of mice was best in those having the H-2^b haplotype.

The dried blood specimens collected on the filter paper were useful and utilized widely in a variety of serologic tests. The antibody on the paper is well maintained under desiccating conditions even at warm temperatures in the Tropics. Serological methods were well developed with high reliability. These included the techniques of gel diffusion, immuno-electrophoresis, circumoval precipitin test, ELISA with sensitive substrate of ABTS, intraoval precipitin test and immuno-fluorescent test using egg and worm antigens, and eggs in formalin-fixed sections. The skin test was not sensitive with either hemoglobin specific protease (HSP) or VBS antigens among schoolchildren infected with *S. mansoni*. Thymocytotoxic autoantibodies were demonstrated in sera of C3H/HeJms, C57BL/6J and ddY mice infected with *S. japonicum* using C57BL/6J thymocytes as target cells. Purification of antigens and their characterization were remarkably advanced. HSP exhibited species specificity in RAST, the egg antigens were heat stable, and the sugars contained were analyzed by lectin bindings.

Clinical observations of acute and chronic stages of *S. japonicum* infection, particularly with cerebral and hepatosplenomegalic manifestations, were made both in Japan and the Philippines, showing characteristic findings in the electroencephalograms which showed hepatocerebral syndrome and paroxysmal attacks in two areas, respectively. The effect of antischistosomal drugs on cerebral involvement was proven, and the mechanism of action of praziquantel to force shedding of miracidium from the egg was proven *in vitro* and *in vivo*.

A species of *Oncomelania* having more antigens commonly shared with a strain of *S. japonicum* was more susceptible to infection by that schistosome; these common antigens had a molecular weight of 70,000 to 90,000.

In *S. mansoni* a *de novo* pathway for pyrimidine biosynthesis was demonstrated. Carbamoylphosphate synthetase was shown to play a key role in the control of the initial steps of pyrimidine synthesis. In mitochondrial fractions of *B. glabrata*, a unique cyanide insensitive pathway was found in addition to the typical cyanide-sensitive one. *In vitro* and *in vivo* studies on the mechanism of molluscicidal action of phebrol showed that the compound probably inhibited the snail succinate-cytochrome-c reductase in both the aerobic and anaerobic respiration.

As for filariasis, the control of both Bancroftian and Malayan filariases was effected in Japan by a successful campaign from 1962 to 1969. The campaign's main weapon consisted in selective mass treatment with diethylcarbamazine (DEC). Improvement of the filtration technique made the surveillance of filariasis more satisfactory and efficient. A formula for indicating microfilarial (mf) density in a population and a formula to describe the periodicity of mf count in the peripheral blood were devised and are effectively describing the local and worldwide epidemiological features.

In epidemiological studies outside of Japan, *Wuchereria bancrofti* in Mindanao, Philippines was of low periodicity showing certain microfilaremia in the daytime. A new endemic area of *Brugia timori* was found on Flores Island, Indonesia. Since 1973, epidemiological studies on onchocerciasis, aiming for control, have been continued at San Vicente Pacaya, Guatemala. The prevalence of mf positives in skin snips was 33.2% and that in the anterior chamber of eye was 6.2%. The annual incidence rates in mf in skin of male and female were 0.064 and 0.035, respectively. In the study of *Onchocerca* nodules, half the nodules harbored only a single female.

Skin tests for onchocerciasis were evaluated and new serologic techniques were developed using crude or defined, homologous or heterologous antigens, sometimes using an extract of dried blood on filter paper. Nevertheless, not one of the serologic methods was as consistently reliable as they were for the diagnosis of schistosomiasis. Antigens, producing specifically IgG and IgE, respectively, were separated from adult *Dirofilaria immitis*, and proportions of proteins and sugars contained in each antigen were determined. Isolation of mf without host cells was successfully performed by enclosing them in 0.4% agarose to allow them to release from the gel.

Vaccination with infective larvae of *B. malayi* and *B. pahangi* attenuated by gamma irradiation protected 93% of the challenged mice against infection. Among a lot of monoclonal antibodies produced against *O. volvulus* (Ov), Ov specific antibodies were isolated.

A sero-epidemiological relationship was found between filaria and HTLV infections in human population in the old filaria infested areas.

Strong acid phosphatase activity was observed histochemically in the subcuticular layer of the body wall in *D. immitis*, *B. pahangi*, and *L. carinii* and at the same site and in mf *in utero* in *Setaria* sp. of the cow. Two types of b-cytochrome were shown to function in the electron transport system of *D. immitis*. The one was oxidizable by fumarate, indicating the presence of an alternative electron transport branch employing fumarate as the hydrogen acceptor. In the study of *B. pahangi* in the jird, estradiol suppressed the susceptibility of the host and retarded the development in the mosquito, whereas progesterone stimulated the development of larval stage in the host.

In morphological studies of mf, the number of nuclei between the cephalic space and the nerve ring was characteristic to each of 6 species. Chromosome numbers of *D. immitis*, *S. digitata*, *O. volvulus*, and *O. gutturosa* and their sex types were clarified.

Various animal models of filariasis were established for pathophysiological and therapeutic studies. Those were adult *B. malayi* implanted intraperitoneally into the jird, *B. pahangi* injected into the GN hamster intratesticularly, *L. carinii* injected in the nude mouse (nu/nu), and mf of *D. immitis* inoculated into mice subcutaneously. The microfilaricidal effect of DEC was investigated in experimentally infected animals to elucidate its mode of action. The results implied that immunological conditioning together with macrophage activity of the host is required for the drug to be active. Output of mf in *D. immitis* cultured *in vitro* was stopped by addition of DEC in the medium. Mf of *B. malayi* in the jird was affected by giving DEC for 5 consecutive days though it required more than a single dose.

Different susceptibilities of *Ae. togoi*, *Ae. albopictus*, *Armigeres subalbatus* and *Culex pipiens pallens* were found for *B. malayi* and *B. pahangi*. Genetic variants and identity have been analyzed by chromosome patterns of anopheline mosquitoes, such as *An. barbirostris* and *An. minimus* groups in Asia and in the Pacific. A strain of *An. hyrcanus* was found to be neither susceptible to *B. malayi* nor to vivax malaria. Inter-crossing among local strains of *Cx. pipiens* complex in China was studied, and most strains were compatible except for females of Fuzhou strain to males of 2 local strains. Susceptibility of blackflies, *Simulium ochraceum*, *S. pintoi*, *S. metallicum*, and *S. haematopotum* to *O. volvulus* was found to differ greatly by locality in Venezuela and Guatemala.

Studies on biology, ecology, and genetics of mosquitoes and blackflies as well as vector snails should lead to a better understanding of the complicated processes in the transmission of the vector-borne diseases concerned and to the development of further improved approaches to their control.

FUTURE GOALS

In spite of the tremendous effort to expand schistosomiasis and filariasis research, full understanding of these diseases is still lacking. Though some control practices have been successful in certain small areas where economical development has progressed to a certain level, large numbers of people in many parts of the world are still greatly affected by these diseases.

Not infrequently, it has been recommended that the panel intensify its efforts to meet the needs of the whole world. The panel is fully aware of the fact that the final goal is to bring these diseases under complete control, and that all research should be oriented to this end. However, the panel is also convinced that the best and the shortest way to reach this goal is to accumulate more basic knowledge to produce new effective tools applicable to disease control.

The Joint Panels met at Kumamoto on 22 and 24, August 1982, and discussed Communique of the 18th Joint Committee Meeting issued on July 23, 1982, which recommended holding a symposium on the current status of parasitic infections in 1983 in place of the 18th Joint Conference. The Symposium on "Current Status of Unresolved Problems in Parasitic Diseases" was realized at Bethesda, Maryland on July 18-19, 1983. Besides the two parasitic diseases, schistosomiasis and filariasis, on which the panels had been concentrating their activities, the present status and problems on the other parasitic diseases such as malaria, trypanosomiasis, leishmaniasis, strongyloidiasis, giardiasis, and *Pneumocystis carinii* pneumonia were reviewed.

After the symposium, the panels drafted revised guidelines that expanded their research to other selected helminthic and protozoal diseases. Special emphasis was placed on vector-borne diseases. Both basic and applied research are supported in the following areas:

Epidemiology
Vector Biology and Control
Parasite Biology
Host-Parasite Interactions
Pathology
Immunology
Biochemistry
Chemotherapy

Thus, the proposed guidelines were submitted for consideration to the Joint Committee meeting held at Bethesda on July 21 and 22, 1983. The new guidelines were approved by the Joint Committee in June 1984 at the meeting held in Tokyo.

The future goals are now on the way to being modified to expand the kinds of parasitic diseases that the panel will study. The activities of the Japanese panel under the new guidelines will begin with the 1985 Japanese fiscal year which starts in April 1985.

During the transitional phase, panels of both countries decided to convene a minisymposium on "Ecology and Control of Vectors" during the 19th Joint Scientific Conference held at Tokyo on August 21-24, 1984, in which topics of mosquitoes, malaria, onchocerciasis, *Onchomelania*, and *Biomphalaria* snails and schistosomiasis were highlighted.

These parasites exist in an extremely complicated and well balanced ecological system in nature. The research will aim to locate and develop tools to attack, in the most effective and feasible way, the vulnerable points in the life cycle of the parasites

SHORT SUMMARY OF THE PAST 20 YEARS

Since 1965, research on schistosomiasis and filariasis has been supported under this program. No newly infected human case of schistosomiasis has been found in Japan since the last one was reported in Kofu in 1977. Control measures were directed exclusively at eliminating the vector snail, *Oncomelania nosophora*, mainly by environmental modification. All snails were eradicated by 1981 except for a few remaining in the Kofu area. Eradication studies are underway in a few foreign countries.

Mass chemotherapy with praziquantel in the Philippines remarkably reduced transmission as measured by incidence. The uneven distribution of the snail, *O. quadrasi*, was studied thoroughly, and the snail survey data were successfully normalized by a simple transformation formula, log (x + 0.01). Studies are being done with phebrol, a synthetic molluscicide, and an extract of *Jatropha curca* seed.

Culture media were improved for the *in vitro* cultivation of *S. japonicum* adults and miracidia, and for midget tissues of *O. hupensis*. The killing effect of monoclonal antibodies of IgG1, IgG2a, and IgE, neutrophils, eosinophils and complement on schistosomula cultured *in vitro* is under study. Daughter sporocysts of *S. mansoni* have been successively transplanted in *B. glabrata*.

Human low responders to schistosomal worm antigen showed a statistical association with HLA-BW52/Dw12/DR2, and the low responsiveness was apparently controlled by specific suppressor T cells. Restriction mapping of the HLA-class II genes implicated five genes, DRβ, DCβ, and three new ones. Development of IgE antibody against *S. japonicum* was best in mice exhibiting the H-2^k haplotype.

Blood added to filter paper and then stored in a dessicator, even at room temperature in the Tropics, was a satisfactory source of antibody. Several serological methods for diagnosing schistosomiasis have been developed that are highly reliable. Hemoglobin specific protease exhibited species specificity in RAST.

Clinical observations on cerebral and hepatosplenomegalic types of schistosomiasis were done in Japan and in the Philippines, and characteristic electroencephalograms were found for both areas.

The etiology of cerebral schistosomiasis was found to be due to the egg embolism and/or vascular changes in the brain. The action of praziquantel on forced shedding of miracidium from the egg was clarified.

In the study on the *de novo* pathway of pyrimidine synthesis in *S. mansoni*, carbamoylphosphate synthetase was found to play a key role in the initial steps. A unique cyanide insensitive pathway was found in a mitochondria fraction of *B. glabrata*.

As for the filariasis research, the control of human filariases was completed in Japan with a campaign from 1962 to 1969 using selective mass treatment with diethylcarbamazine (DEC). Improvement of the filtration technique for microfilaria (MF) detection made the survey efficient and satisfactory. The methods of data analysis for mf density and periodicity in a locality were devised for characterizing the local epidemiological features.

Since 1973, epidemiological studies were performed on onchocerciasis in Guatemala. The prevalence of mf in the skin and eyes was 32.2% and 6.2%, respectively. The annual mf incidence observed in the skin was 6.4% and 3.5% in males and females, respectively. Serologic methods for diagnosis were advanced fairly well using various antigens. Antigens derived from adult *D. immitis*, specifically IgG and IgE, were separated and their chemical characteristics were analyzed.

Vaccination with infective larvae attenuated by gamma irradiation protected 93% from the challenge infection. Among monoclonal antibodies against *O. volvulus*, specific antibodies were isolated. A seroepidemiological relationship was found between the filaria and ATLV infections in the old filaria infested areas in Japan.

Of two types of b-cytochrome functioning in the electron transport system of *D. immitis*, one was oxidizable by fumarate. This indicated the presence of an alternate electron transport system employing fumarate as the hydrogen acceptor.

Several animal models of filariasis were established which strengthened pathophysiological and therapeutic studies. The results of studies on the mechanism of action of DEC indicated that immunological conditioning together with macrophage activity was required for the drug to be active.

For a few groups of anopheline mosquitoes genetic variants have been identified and analyzed by chromosome patterns. Studies of the biology, ecology, and genetics of vector insects and snails should lead to a better understanding of transmission dynamics of filariasis and schistosomiasis, and facilitate their control.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
Dr. Yoshiki Aoki Nagasaki University	Epidemiology of filariasis and schistosomiasis
Dr. Keizo Asami Keio University	Biochemistry of schistosomiasis
Dr. Koichiro Fujita Nagasaki University	Immunology of filariasis

Investigator and Institution	*Title*
Dr. Shigeo Hayashi National Institute of Health	Epidemiology and immunology of filariasis and schistosomiasis
Dr. Seiiti Inatomi Okayama University	Ultrastructure of schistosomiasis and filariasis
Dr. Akira Ishii Okayama University	Immunology of schistosomiasis
Dr. Tozo Kanda St. Marianna University	Epidemiology of filariasis and biology of the vector of filariae
Dr. Kenjiro Kawashima Kyushu University	Biology and control of vector snails of schistosome
Dr. Somei Kojima Shinshu University	Immunology of schistosomiasis
Dr. Takeshi Kurihara Teikyo University	Biology of mosquitoes
Dr. Toshio Nakabayashi Ohsaka University	Biology of malariae
Dr. Munetoshi Niimura Chiba University	Epidemiology and chemotherapy of schistosomiasis
Dr. Yoshito Otsuji Kagoshima University	Epidemiology and chemotherapy of filariasis
Dr. Hiroshi Oya Juntendo University	Biochemistry of schistosomiasis
Dr. Takehiko Sasazuki Tokyo Medical & Dental University	Immunogenetics of schistosomiasis
Dr. Atsuo Sato Kagoshima University	Immunodiagnosis and epidemiology of filariasis and schistosomiasis
Dr. Kumiko Sato Gunma University	Immunochemistry of filariasis and schistosomiasis
Dr. Toshiro Shibuya Teikyo University	Immunology and chemotherapy of filariasis
Dr. Mamoru Suzuki Gunma University	Studies on malaria vaccine
Dr. Isao Tada Kumamoto University	Immunodiagnosis and epidemiology of filariasis and schistosomiasis
Dr. Suehisa Takada Ohsaka City University	Biology of haemoprotozoa and filariae
Dr. Hiroshi Tanaka University of Tokyo	Epidemiology, immunology, and chemotherapy of schistosomiasis and filariasis

Investigator and Institution	*Title*
Dr. Moriyasu Tsuji Hiroshima University	Immunodiagnosis of schistosomiasis, laboratory breeding of oncomelania snails
Dr. Hiroshi Tsutsumi Kurume University	Pathology and immunology of schistosomiasis
Dr. Yoshito Wada National Institute of Health	Biology and control of vectors of filariae
Dr. Hisashi Yamamoto Dokkyo University	Biology of vectors of filariae
Dr. Toshio Yanagisawa Kitasato University	Biochemistry of schistosomiasis and filariasis
Dr. Kazuo Yasuraoka University of Tsukuba	Biology and control of schistosomiasis
Dr. Muneo Yokogawa Chiba University	Epidemiology, immunology, and chemotherapy of schistosomiasis

BIBLIOGRAPHY

Aoki, M., A.L. Vincent, L.R. Ash, and D. Katamine. Scanning electron and microscopy of third- and fourth-stage larvae and adults of *Brugia pahangi* (Nematoda:Filarioidea). *J Parasitol.* *66*:449–457, 1980

Aoki, Y., M.M. Recinos, and Y. Hashiguchi. Life span and distribution of *Onchocerca volvulus* microfilariae in mice. *J Parasitol.* *66*:797–801, 1980

Aoki, Y., M. Sakamoto, T. Yoshimura, I. Tada, M.M Recinos, and H. Figueroa. Onchocercomas in Guatemala, with special reference to appearance of new nodules and parasite content. *Am. J. Trop. Med. Hyg.* *32*:741–746, 1983

Amano, T. Clinicopathological studies on the gastro-intestinal schistosomiasis in the endemic area of Yamanashi prefecture, with special reference to the carcinogenicity of schistosome infection. *Jap. J. Parasit.* *29*:305–312, 1980 (text in Japanese)

Amano, T. Clinicopathological studies on the gastro-intestinal schistosomiasis in the endemic area of Yamanashi prefecture, with special reference to the carcinogenecity of schistosome infection. 2. Histopathological studies on the gastro-duodenal lesions with schistosome eggs. *Jap. J. Parasit.* *30*:135–149, 1981 (text in Japanese)

Amano, T. Clinicopathological studies on the gastro-intestinal schistosomiasis in the endemic area of Yamanashi prefecture, with special reference to the carcinogenecity of schistosome infection. 3. Histopathological studies on the colorectal lesions with schistosome eggs. *Jap. J. Parasit.* *31*:9–21, 1982 (text in Japanese)

Amano, T. and T. Oshima. Thymic atrophy in experimental murine schistosomiasis japonica. *Jap. J. Parasit.* *32*:591–595, 1983

Amano, T. and T. Oshima. Hepatosplenomegaly and changes of blood pictures in mice infected with *Schistosoma japonicum* *Yokohama Med. Bull.* *35*:43–54, 1984

Anantaphruti, M., M. Terada, A.I., Ishii, H. Kino, M. Sano, M. Kuroyanagi, and S. Fukushima. Studies on Chemotherapy of Parasitic Helminths (XI). *In Vitro* Effects of Various on the Motor Activity of Adult *Schistosoma japonicum*. *Jap. J. Parasit* *31*:321–328, 1982

Anantaphruti, M., H. Kino, M. Terada, A.I. Ishii, and M Sano. Studies on Chemotherapy of Parasitic Helminths (XIII) Efficacy of Ivermectin on the Circulating Microfilaria and Embryonic Development in the Female Worm on *Dirofilaria immitis*. *Jap. J. Parasit.* *31*:517–529, 1982

Asahi, H., M. Kawabata, F. Sendo, M. Naiki, Y. Hosaka, and T. Kobayakawa. The presence of anti-sheep red blood cell heterophile antibodies and their characteristics in murine schistosomiasis japonica. *Microbiol. Immunol.* *28*:1241–1256, 1984

Asahi, H., A. Moribayashi, F. Sendo, and T. Kobayakawa Hemolytic factors in *Schistosoma japonicum* eggs. *Inf. Immun* *46*:514–518, 1984

Asahi, H., M. Kawabata, F. Sendo, M. Naiki, and T Kobayakawa. Characteristics of the anti-sheep red blood cell heterophile antibodies produced during *Schistosoma japonicum* infection in mice. *Int. Archs. Allergy Appl. Immun.* *13*:1985, in press

Asami, K. and M. Tanabe. Biochemical characterization of the granulomatous reaction around *Schistosoma mansoni* eggs in the tissue. *Jpn. J. Trop. Med. Hyg.* *9*:109–110, 1981, suppl

Asami, K., M. Tanabe, T. Takeuchi, S. Miura, and S Kobayashi. Influence of splenectomy on the liver fibrosis in the mice infected with *Schistosoma mansoni* *Jpn. J. Parasit.* *31*:7, 1982, suppl

Beisler, G.K., H Matsuda, M Nakao, and H Tanaka. Variations of titers of immunofluorescent antibody against cercaria, adult and egg antigens developed in rabbits infected with *Schistosoma japonicum* before and after treatment with praziquantel. *Japan. J. Exp. Med.* *54*:17–22, 1984

Beisler, G.K., H Matsuda, M Nakao, and H Tanaka Identity of antigens in adult tegument, gut, egg and cercaria of *Schistosoma japonicum* by immunofluorescent study. *Japan. J Exp. Med.* *54*:125–130, 1984

Beisler, G.K., M. Nakao, H. Matsuda, and H. Tanaka Characterization of the carbohydrates of *Schistosoma japonicum* adult worm, egg, and cercaria by analysis of lectin binding and antibody reaction. *Japan. J. Exp. Med.* *54*:263–273, 1984

Chigusa, Y., H. Tsuruoka, N. Ogura, and M Kobayashi. An application of the thin layer gel-electrophoresis for the analysis of esterase isozyme in the mosquitoes, *Aedes aegypti*. II Esterase isozymes of two susceptible and two refractory strains of *Aedes aegypti* to *Brugia malayi*. *J. Aichi Med. Univ. Assoc.* *11*:386–410, 1983

Fujita, K. and S. Tsukidate. Preparation of a highly purified allergen from *Dirofilaria immitis*. *Immunology* *42*:363–370, 1981

Fujita, K., S. Tsukidate, and T. Ikeda. *Dirofilaria immitis*: Physicochemical properties of IgG-inducing antigen with special reference to the comparison with highly purified allergen. *Trop Med.* *23*:193–204, 1981

Fujita, K. and S. Tsukidate. Localization of highly purified allergen and IgG-inducing antigen in adult *Dirofilaria immitis* determined by fluorescent antibody test. *Trop. Med* *24*:219–227, 1982

Fujita, K. and S. Tsukidate. Single radial immunodiffusion method for determination of the allergen concentration in *Dirofilaria immitis*. *Trop. Med.* *25*:165–168, 1983

Fujita, K., K. Tajima, S. Tsukidate, T. Oda, K. Kurokawa, L. Moncada, M. Ueda, A. Mori, and Y. Hinuma. Correlation filarial antibody titre and adult T-cell leukemia virus antibody titre in inhabitants of Goto Islands, Nagasaki, Japan. *Japan J.T.M.H.* *11*:225-233, 1983 (text in Japanese)

Fujita, K. and S. Tsukidate. Allergen concentration in *Dirofilaria immitis*. *J. Parasit.* *70*:313-314, 1984

Fujita, K., T. Ikeda, and S. Tsukidate. Amino acid composition of the highly purified allergen from *Dirofilaria immitis*. *Int. Archs. Allergy Appl. Immunol.* *73*:184-185, 1984

Fujita, K. and T. Kurihara. Oviposition regulation of methoprene in *Culex pipiens molestus*. *Jap. J. Sanit. Zool.* *35* (in press) 1984. (Text in Japanese.)

Fujita, K. and S. Tsukidate. A highly purified allergen from excretory and secretory products of *Dirofilaria immitis*. *Int. J. Parasit.* *14*: in press, 1984

Hashiguchi, Y., I. Tada, M.M. Recinos, J.O. Ochoa, and P.A. Molina. Bovine and equine onchocerciasis in Guatemala, especially in San Vicente Pacaya. *J. Parasitol.* *67*:286-287, 1981

Hashiguchi, Y., M. Kawabata, M. Takaoka, T. Yoshimura, and M.M. Recinos. The long-term absence of onchocerciasis in an area where the vectors, Simulium spp., are found. *Trans. R. Soc. Trop. Med. Hyg.* *75(6)*:*901*, *1981*

Hashiguchi, Y., I. Tada, O. Flores, C., and H. Takaoka. Diurnal biting activity of four zoophilic species of Simulium in an area endemic for human onchocerciasis in Guatemala. *Jap. J. Trop. Med. Hyg.* *10*:239-244, 1982

Hayashi, M., H. Hirasawa, and M. Kamaki. Schistosomiasis mansoni — A problem of imported parasite disease — *Nihonijishinpo* *2945*:26-30, 1980 (text in Japanese)

Hayashi, M., T. Kumakura, L.C. Tormis, and J.S. Nosañas. Effect of antischistosomal agent (praziquantel) on hepatosplenomegalic type of schistosomiasis japonica in Leyte Island, Philippines. Yamanashi Med. Assoc. 1:1-7, 1980 (text in Japanese)

Hayashi, M. Relationship between acute or chronic schistosomiasis japonica and brain function— from the point of view in clinical symptoms and electroencephalographic findings— Battle against schistosomiasis japonica (Chihobyo tono tatakai) 208-247. *Yamanashi Pref.* *1981* (text in Japanese)

Hayashi, M., M. Kamaki, H. Hirasawa, L.C. Tormis, J.S. Nosañas, and B.L. Blas. Clinical study on chronic schistosomiasis japonica in Leyte Island— Effect of antischistosomal agent on cerebral and hepatosplenomegalic type of schistosomiasis japonica. *Yamanashi Med. Assoc.* 8:4-9, 1981

Hayashi, M., M. Kamaki, and S. Shintani. Clinical study on cerebral schistosomiasis japonica in Leyte Island, Philippines. Follow-up study 5 years after treatment. *Yamanashi Med. Assoc.*

Hayashi, M., M. Kamaki, S. Shintani, L.C. Tormis, J.S. Nosañas, and B.L. Blas. Clinical study on chronic schistosomiasis japonica in Leyte Island, Philippines. *Yamanashi Med. Assoc.* *10*:11-18, 1983

Hayashi, M., S. Shintani, S. Motohashi, L.C. Tormis, J.S. Nosañas, B.L. Blas. Follow-up study on cerebral schistosomiasis japonica 7 years after treatment in Leyte Island, Philippines. *Yamanashi Med. Assoc.* *11*:1-7, 1984

Hayashi, M., S. Shintani, S. Minai, and T. Horimi. On the changes in clinical features of subjective type (light infection type) of schistosomiasis japonica—Follow-up study in the endemic area of schistosomiasis japonica for 17 years— *Yamanashi Med. Assoc.* *12*:8-14, 1984

Hayashi, Y., S. Nogami, and M. Nakamura. Comparison of activities of mononuclear phagocyte system between the cotton rat, *Sigmodon hispidus*, and the jird, *Meriones unguiculatus* in relation to the treatment of filariasis. *Japan. J. Exp. Med.* *53*:257-262, 1983

Hayashi, Y., S. Nogami, and T. Shibuya. Effect of stimulation and blockade of mononuclear phagocyte system on the treatment of microfilaremia of *Litomosoides carinii* in the cotton rat and the jird. *Japan. J. Exp. Med.* *54*:51-55, 1984

Hayashi, Y., A. Sadanaga, A. Shirasaka, S. Nogami, and H. Tanaka. High ratio of survivals and stable recovery of adult *Brugia malayi* transplanted into the jird, *Meriones unguiculatus*. *Japan. J. Exp. Med.* *54*:87-89, 1984

Hayashi, Y., K. Noda, A. Shirasaka, S. Nogami, and M. Nakamura. Vaccination of BALB/c mice against *Brugia malayi* and *B. pahangi* with larvae attenuated by gamma irradiation. *Japan. J. Exp. Med.* *54*:177-181, 1984

Hayashi, Y., S. Nogami, M. Nakamura, A. Shirasaka, and K. Noda. Passive transfer of protective immunity against *Brugia malayi* in BALB/c mice. *Japan. J. Exp. Med.* *54*:183-187, 1984

Hida, M.M., J.J. Ferraroni, H.V. Dourado, K. Sato, and M. Suzuki. Skin test with purified Sawada antigen in Brazilian Indians with ocular onchocerciasis. The Fourth Japan-Brazil Symposium on Science and Technology, *Proceedings of Symposium*, 106-112, 1984

Hirata, M. Female-dependency of circulating anodic antigen level in *Schistosoma japonicum* infection. *Jap. J. Parasit.* *30*:429-437, 1981

Hirata, M., Y. Yoneda, Y. Takao, H. Iida, K. Miyasaka, N. Yoshioka, K. Maki, and H. Tsutsumi. Prevalence of antibodies to *Schistosoma japonicum* in the Chikugo River basin. *Kurume Med. J.* *28*:227-232, 1981

Hirata, M., M. Miyasato, and H. Tsutsumi. Increased reactivity of *Schistosoma japonicum* eggs in the circumoval precipitin test after hydrochloric acid treatment. *Kurume Med. J.* *31*:65-71, 1984

Hosaka, Y., H. Asahi, T. Ishizaki, M. Tanabe, F. Senda, and T. Okuda. The intradermal test for the survey of schistosomiasis in cattle. *Jap. J. Parasit.* 29:203–208, 1980.

Hosaka, Y., J. Hashiguchi, Y. Sakata, E.A. Banez, and B.L. Blas. An assessment of the molluscicidal activity of B-2 and some other chemicals against *Oncomelania quadrasi*. *Jap. J. Parasit.* 33:55–58, 1984.

Hunter, G.W., III, M. Yokogawa, M. Akusawa, M. Sano, K. Araki, and M. Kobayashi. Control of schistosomiasis japonica in the Nagatoshi area of Kurume, Japan. *Am. J. Trop. Med. Hyg.* 31:760–770, 1982.

Hunter, G.W., III, and M. Yokogawa. Control of schistosomiasis japonica in Japan. A review - 1950–1978. *Jpn. J. Parasit.* 33:341–351, 1984.

Inokuchi, T. Pulmonary lesions in schistosomiasis japonica. I. Human autopsy cases. *J. Kurume Med. Assn.* 42:1082–1100, 1979 (text in Japanese).

Inokuchi, T. Pulmonary lesions in schistosomiasis japonica. II. Experimental animal cases. *J. Kurume Med. Assn.* 42:1101–1115, 1979 (text in Japanese).

Inokuma, S., S. Sasaki, T. Shibuya, and K. Araki. A case of tropical eosinophilia supposedly caused by *Dirofilaria immitis*. *Allergy* 30:1071–1076, 1981.

Inokuma, S., S. Sasaki, T. Shibuya, and K. Araki. Tropical eosinophilia presumably due to *Dirofilaria immitis* infection. *Jpn. J. Allergol.* 30:1071–1076, 1981 (text in Japanese).

Irie, Y. and K. Yasuraoka. *Schistosoma japonicum*: ultrastructural changes in the tegument during cercaria-schistosomulum transformation. *Japan. J. Exp. Med.* 51:53–63, 1981.

Irie, Y., and K. Yasuraoka. Morphological alterations of *Schistosoma japonicum* associated with the administration of amoscanate. *Japan. Exp. Med.* 52:139–148, 1982.

Irie, Y., P.F. Basch and N. Basch. Reproductive ultrastructure of adult *Schistosoma mansoni* grown in vitro. *J. Parasitol.* 69:559–566, 1983.

Ishii, A. and M. Owhashi. Enzyme-linked immunosorbent assay with egg antigens of *Schistosoma japonicum*. *Zeitschrift fur Parasitenkunde*. 67:279–287, 1982.

Ishii, A., M. Owhashi, and I. Hashiguchi. *Schistosoma japonicum*: Immediate hypersensitivity reactions to egg antigen in humans and rodents. *Exp. Parasit.* 54:104–112, 1982.

Ishii, A., B.D. Cabrera, S. Sugiura, M. Kobayashi, T.G. Go and F. Valeza. An epidemiological study of filariasis in Sorsogon Province, Republic of the Philippines, with notes on experimental mosquito infection. *J. Trop. Med. Hyg.* 86:59–64, 1983.

Ito, Y. Review of parasitological, epidemiological, and immunological investigation in onchocerciasis control project in Guatemala, 1976–1980. *ORCOP Publications No. 43*, 1981.

Iwanaga, Y. The growth of young snail and fecundity of *Oncomelania hupensis hupensis* in the laboratory. *Jap. Jour. Malac.* 39:63–68, 1980 (text in Japanese).

Iwanaga, Y. and M. Tsuji. Observation on the infection of *Oncomelania* spp. to *Schistosoma japonicum* (5). The susceptibility of laboratory colonies of *Oncomelania hupensis hupensis* to *Schistosoma japonicum*, Chinese strain. *Med. J. of Hiroshima Univ.* 30:787–790, 1982 (text in Japanese).

Iwanaga, Y. and M. Tsuji. Observation on the infection of *Oncomelania* spp. to *Schistosoma japonicum* (6). The susceptibility of laboratory colonies of *Oncomelania* spp. of the different geographical strains to *Schistosoma japonicum*, Chinese strain. *Med. J. of Hiroshima Univ.* 30:791–796, 1982 (text in Japanese).

Iwanaga, Y. and M. Tsuji. Studies on host-parasite relationship of *Schistosoma japonicum*. *Med. J. of Hiroshima Univ.* 31:479–483, 1983 (text in Japanese).

Iwanaga, Y., H. Shimomura, S. Katayama, and M. Tsuji. Observation on infection of *Oncomelania* spp. to *Schistosoma japonicum* (7). The infectivity of laboratory colonies of *Oncomelania hupensis hupensis* to Yamanashi and Leyte strains of *Schistosoma japonicum*. *Jap. J. Parasit.* 33:23–27, 1984 (text in Japanese).

Iwanaga, Y. and M. Tsuji. Studies on host-parasite relationship between *Schistosoma japonicum* and *Oncomelania* snails. (1). Antigenic communities between the Chinese strain of *Schistosoma japonicum* adult worm and *Oncomelania* snails. Jap. J. Parasit. 34, (in press), 1985.

Kaji, R., T. Kamijo, A. Yano, and S. Kojima. Genetic control of immune responses to *Schistosoma japonicum* antigen. *Parasite Immunology* 5:25–35, 1983.

Kakizoe, Y., M. Hirata, Y. Yoneda, H. Iida, S. Yoshioka, M. Miyasato and H. Tsutsumi. The distribution of *Oncomelania nosophora* in the Chikugo River basin in the last five years. *J. Kurume Med. Assn.* 46:902–911, 1983 (text in Japanese).

Kamiya, H., Y. Tada, H. Matsuda, H. Tanaka, B.L. Blas, J.S. Nosenas, and A. T. Santos, Jr. Annual fluctuation of *Schistosoma japonicum* infection in field rats, *Rattus rattus mindanensis*, in Dagami, Leyte, Philippines. *Japan. J. Exp. Med.* 50:375–382, 1980.

Kamiya, H. Immunodiagnosis of schistosomiasis by the application of whole parasites or parasite tissue sections, a review. *Akita J. Med.* 7:331–343, 1981.

Kamiya, H., T. Suzuki, H. Matsuda, and H. Tanaka. Diagnosis of schistosomiasis japonica by indirect immunoperoxidase technique using *Schistosoma japonicum* eggs in formalin fixed liver sections. *Jpn. J. Parasit.* 30:587–596, 1981.

Kamiya, H. Technique of the intraoval precipitin (IOP) reaction by using formalin fixed tissue section for the diagnosis of schistosomiasis. *Jpn. J. Parasit.* *30*:161-165, 1981

Kamiya, H., T. Suzuki, H. Matsuda, and H. Tanaka. Evaluation of an indirect fluorescent antibody test (IFAT) on formalin-fixed liver-egg sections for the diagnosis of schistosomiasis. *Southeast Asian J. Trop. Med. Pub. Hlth.* *13*:249-256, 1982

Kamiya, H. Circumoval precipitin (COP) test using air-dried eggs of *Schistosoma japonicum* and *S. mansoni*. *Southeast Asian J. Trop. Med. Pub. Hlth.* *14*:451-455, 1983

Kamiya, H. and B.L. Blas. Application of formalin-fixed *Schistosoma japonicum* eggs to the circumoval precipitin test. *Z. Parasitenkd.* (in press)

Kamiya, H., K. Yamashita, S. Ishigooka, K. Yoshimura, and B.L. Blas. Enhancement of circumoval precipitin reactivity by sonication in the paraformaldehyde-fixed eggs of *Schistosoma mansoni* and *S. japonicum*. *Jpn. J. Parasit.* (in press)

Kamo, E. Symptomatology of acute schistosomiasis japonica. *In* The battle against endemic disease in Yamanashi Prefecture—Medical aspects of schistosomiasis japonica—ed by Yokogawa, M., S. Hayashi, T. Iijima. *Yamanashi Schistosomiasis Control Association, Kofu*: 106-135, 1981 (text in Japanese)

Kamo, E. and T. Ebato. A clinical analysis of primary carcinoma of the liver in relation with schistosomiasis japonica. *J. Yamanashi Med. Assoc. (Yamanashi Igaku)* 9:156-170, 1982 (text in English)

Kanda, T., G.L. Chiang, K. Ito, W. Fumurasvin, and K.P. Loong. Cytogenetic observations on *Anopheles dirus* of the leucosphyrus complex. *Mosquito News* *40*:585-592, 1980

Kanda, T. Recent developments in mosquito taxonomy. Recent advances in Entomology pp. 588 ed by Ishii, S. Univ. Tokyo Press, 1981 (in Japanese)

Kanda, T., K. Takai, Y. Oguma, G.L. Chiang, W.H. Cheong, S. Sucharit, and A.M. Joesoef. Evolutionary genetics of the *Anopheles hyrcanus* group, the leucosphyrus group and the Pyrethophorus group in East Asia and Pacific area. *Cytogenetics and Genetics of Vectors* Ed. Pal, Kitzmiller and Kanda Kodansha-Elsevier, 31-60, 1981

Kanda, T., K. Takai, G.L. Chiang, W.H. Cheong, and S. Sucharit. Hybridization and some biological facts of seven strains of the Anopheles leucosphyrus group (Reid, 1968). *Jpn. J. Sanit. Zool.* *32*:321-329, 1981

Kanda, T., W.H. Cheong, Y. Oguma, K. Takai, G.L. Chiang and S. Sucharit. Systematics and cytogenetics of the *hyrcanus* group, *leucosphyrus* group, and Pyrethophorus group in East Asia. *Recent development in the Genetics of Insect Disease Vectors*. Stipes Publishing Company, 1982

Kanda, T., K. Takai, G.L. Chiang, K.P. Loong, S. Sucharit, W.H. Cheong. Phylogenetic interpretation and chromosomal polymorphism among nine strains of human malaria vectors of the *Anopheles leucosphyrus* group. *Jpn. J. Genet.* 58:193-208, 1983

Kanda, T. and S. Sucharit. Recent developments in malaria vector biology and epidemiology in East Asia. *Jpn. J. Trop. Med. & Hyg.*: (in press), 1984

Kanda, T., K. Ogawa, G.L. Chiang, S. Sucharit. Cytogenetic and hybridization studies among three strains morphologically variated and belonging to *Anopheles minimus* Theobald from Japan and Thailand (accepted in 1983, and waiting publication)

Kawabata, M., Y. Hosaka, M. Kumada, N. Matsui, and T. Kobayakawa. Thymocytotoxic autoantibodies found in mice infected with *Schistosoma japonicum*. *Inf. Immun.* 32:438-442, 1981

Kawabata, M., I. Tada, Y. Aoki, Y. Hashiguchi, T. Yoshimura, M. Sakamoto, G. Zea F., M.M. Recinos, and C. Flores, O.F. Parasitological diagnosis of onchocerciasis in Guatemala. *Proceedings of the Guatemala-Japan Joint Conference on Onchocerciasis Research and Control.* 144, 1982

Kawabata, M., G.Z. Flores, S. Izui, and T. Kobayakawa. IgM rheumatoid factors in Guatemalan onchocerciasis. *Trans. Royal Soc. Trop. Med. Hyg.* *78*:356-358, 1984

Kawanaka, M. and B.L. Blas. A technique for sero-diagnosis of *Schistosoma japonicum* infection by the indirect fluorescent antibody (IFA) test. *Proceedings of the Philippine-Japan joint conference on schistosomiasis research and control.* 40-42, 1980

Kawanaka, M. Deposition and maturation of *Schistosoma japonicum* eggs in vitro: Comparison between Japanese and Philippine strain. *Jpn. J. Parasit.* 32:305-315, 1983 (text in Japanese)

Kawanaka, M., S. Hayashi and H. Ohtomo. Localization of antigen within eggs of *Schistosoma japonicum* that participate in circumoval precipitin (COP) reaction. *J. Parasit.* *69*:444-446, 1983

Kawanaka, M., S. Hayashi, and H. Ohtomo. Nutritional requirements of *Schistosoma japonicum* eggs. *J. Parasit.* *69*:85[illegible]-[illegible]61, 1983

Kawanaka, M., S. Hayashi, and H. Ohtomo. A minimum essential medium for cultivation of *Schistosoma japonicum* eggs. *J. Parasit.* *69*:991-992, 1983

Kawashima, K. *Schistosoma japonicum*: its intrasnail development, snail host responses and antagonism between *S. japonicum* and other parasites. *Proc. 14th Seminar on Trop. Med.* *IV*:28, 1-3, 1983

Kawashima, K., B.L. Blas, and A.T. Santos, Jr. Experimental infection of *Oncomelania quadrasi* with *Paragonimus ohirai*. *Southeast Asian J. Trop. Med. Pub. Hlth.* *15*:148-154, 1984

Kawashima, K., B.L. Blas, and A.T. Santos, Jr. The cercarial emergence of *Schistosoma japonicum* from *Oncomelania quadrasi* under outdoor conditions in the Philippines. To be published in *J. Helminthol.*

Kimura, E., Y. Nakajima, and Y. Aoki. Enzyme histochemistry of *Brugia pahangi*. 1. Localization of acid phosphatase activity in developing larvae in mosquito (*Aedes aegypti*). *Japan J. Trop. Med. Hyg.* *11*:7-15, 1983.

Kimura, E., Y. Aoki, Y. Nakajima, and M. Niwa. Plasma level of diethylcarbamazine in jirds and hamsters. *Southeast Asian J. Trop. Med. Pub. Hlth.* *15*:74-79, 1984.

Kimura, E., L. Penaia, and G.F.S. Spears. Comparison of methods for the detection of microfilariae of *Wuchereria bancrofti* in Western Samoa. *Southeast Asian J. Trop. Med. Pub. Hlth.* *15*:167-174, 1984.

Kimura, E., S. Shigeno, M. Sakamoto, M. Shimada, Y. Aoki, and J. Waikagul. Studies on *Brugia pahangi* in inbred hamsters. 2. The intratesticular inoculation of infective larvae into CBN hamsters and some other rodents. *Tropical Medicine* 26:51-56, 1984.

Kobayashi, M., N. Ogura, and H. Yamamoto. Studies on filariasis III. Susceptibility of several mosquito species to periodic Che-Ju (Korea) strain of *Brugia malayi*. *Japan. J. Sanit. Zool.* *32*:293-299, 1981.

Kobayashi, M. and H. Yamamoto. Mosquito hemocytes. *Akaieka Newsletter* 9:13-17, 1984.

Kojima, S., A. Yano, T. Sasazuki, and N. Ohta. Association between HLA and immune responses in individuals with chronic schistosomiasis japonica. *Trans. Roy. Soc. Trop. Med. Hyg.* 78:325-329, 1984.

Korenaga, M., I. Tada, T. Mimori, M. Sakamoto, A. Lujan T., G.E. Zea F., J.C. Castro, and L. Yarzabal. Enzyme-linked immunosorbent assay (ELISA) in the detection of IgG antibodies in onchocerciasis using blood collected on filter paper. *Jap. J. Parasitol.* *32*:347-355, 1983.

Kurihara, T. and R. Maeda. Entomological factors affecting the transmission of mosquito-borne filariasis. *Jap. J. Sanit. Zool.* *31*:231-242, 1980 (text in Japanese).

Kurihara, T. and R. Maeda. Observations on the development of the nematode parasite *Romanomermis culicivorax* in pupal and adult *Culex pipiens molestus* mosquitoes. *Mosquito News* *40*:643-645, 1980.

Kurihara, T. Study on Malayan filariasis in Panitian, the Philippines. *Nettai.* *15*:57-63, 1982 (text in Japanese).

Kurihara, T. Post World War II mosquito control in Japan, in "Integrated mosquito control methodologies." Vol. I. *Academic Pr.* 105-134, 1983.

Kurihara, T. Mosquitoes occurring in the plant axils in tropical Asia. *Jap. J. Sanit. Zool.* *35*:163-169, 1984 (text in Japanese).

Kurihara, T., K. Fujita, and T. Suzuki. Insecticide treatment of wide-mesh net curtain for vector control and the effect upon behavioural responses of adult mosquitoes. *Jap. J. Sanit. Zool.* *36*: (in press) 1985 (text in Japanese).

Lai, J.S. Effect of dichlorvos on spermatogenesis of *Armigeres subalbatus*. *Okayama Igakkai Zasshi* *94*:743-753, 1982 (in Japanese).

Lujan Trengay, L.A., M. Sakamoto, M. Shimada, E. Kimura, and Y. Aoki. The change of the indirect hemagglutination titers in mice infected with *Dirofilaria immitis* microfilariae following the administration of diethylcarbamazine. *Tropical Medicine* 26:11-15, 1984.

Maeda, R. and T. Kurihara. The effect of age of *Aedes togoi* on the transmission of *Brugia malayi* in the laboratory. *Japan J. Sanit. Zool.* *31*:277-281, 1980 (text in Japanese).

Maeda, R. and T. Kurihara. New method for measuring the blood volume and the number of microfilariae ingested by individual mosquitoes. *Japan. J. Sanit. Zool.* *31*:299-302, 1980.

Maeda, S., Y. Irie, and K. Yasuraoka. Resistance of mice to secondary infection with *Schistosoma japonicum*, with special reference to neutrophil enriched response to schistosomula in the skin of mice. *Japan. J. Exp. Med.* 52:111-118, 1982.

Maeda, S., Y. Irie, and K. Yasuraoka. Comparison of protein composition between schistosomula of *Schistosoma japonicum* and *S. mansoni*. *Parasitology* *89*: 1984 (in press).

Maki, J. and T. Yanagisawa. Acid phosphatase activity demonstrated in the nematodes, *Dirofilaria immitis* and *Angiostrongylus cantonensis* with special reference to the characters and distribution. *Parasit.* *80*:23-38, 1980.

Maki, J. and T. Yanagisawa. A comparison of the sites of acid phosphatase activity in an adult filaria, *Setaria* sp. and in some gastro-intestinal nematodes. *Parasit.* *81*:603-608, 1980.

Maki, J. and T. Yanagisawa. Histochemical studies on acid phosphatase of the body wall and intestine of adult filarial worms in comparison with that of other parasitic nematodes. *J. Helminth.* *54*:39-41, 1980.

Maki, J., A. Furuhashi, and T. Yanagisawa. The activity of acid proteases hydrolysing haemoglobin in parasitic helminths with special reference to interspecific and intraspecific distribution. *Parasit.* *84*:137-147, 1982.

Makiya, K. Studies on the distribution pattern and the evaluation method of control measure of the intermediate host snail *Oncomelania quadrasi* of *Schistosoma japonicum* in the Philippines. 1. Distribution pattern of the snail, necessary sample size for density survey and significance test of snail density. *Jpn. J. Parasit.* *29*:293-304, 1980 (text in Japanese).

Makiya, K. Studies on the distribution pattern and the evaluation method of control measure of the intermediate host snail *Oncomelania quadrasi* of *Schistosoma japonicum* in the Philippines. 2. Evaluation of the control measure of the host snail by drainage. *Jpn. J. Parasit.* 29:359-368, 1980 (text in Japanese)

Makiya, K., H. Tanaka, E.A. Bañez, B.L. Blas, N. Kumada, and A.T. Santos, Jr. Population studies on *Oncomelania quadrasi*, the snail intermediate host of *Schistosoma japonicum* in the Philippines. 1. Distribution pattern of the snail in the field. *Japan. J. Exp. Med.* 51:179-185, 1981

Makiya, K., H. Tanaka, E.A. Bañez, N. Kumada, and A.T. Santos, Jr. Population studies on *Oncomelania quadrasi*, the snail intermediate host of *Schistosoma japonicum* in the Philippines. 2. Necessary sample size for snail density survey. *Japan. J. Exp. Med.* 51:331-334, 1981

Makiya, K., H. Tanaka, E.A. Bañez, B.L. Blas, N. Kumada, and A.T. Santos, Jr. Population studies on *Oncomelania quadrasi*, the snail intermediate host of *Schistosoma japonicum* in the Philippines. 3. Data transformation for significance test of snail density. *Japan. J. Exp. Med.* 52:33-37, 1982

Makiya, K., H. Tanaka, N. Kumada, E.A. Bañez, B.L. Blas, A. Perez, and A.T. Santos, Jr. Population studies on *Oncomelania quadrasi*, the snail intermediate host of *Schistosoma japonicum* in the Philippines. 4. Evaluation of drainage for snail control. *Japan. J. Exp. Med.* 52:173-181, 1982

Matsuda, H., M. Nakao, H. Tanaka, T. Nagata, J.S. Noseñas, B.L. Blas, G.P. Portillo, and A.T. Santos, Jr. A study of ELISA for *Schistosomiasis japonica* using 5-aminosalicylic acid, a substrate of peroxidase-labeled antibody. *Jpn. J. Parasit.* 30:363-372, 1981 (text in Japanese)

Matsuda, H., and H. Tanaka. Serodiagnosis of schistosomiasis japonica. *Clinical Bacteriology* 8:46-50, 1981 (text in Japanese)

Matsuda, H., H. Tanaka, M. Nakao, B.L. Blas, J.S. Noseñas and A.T. Santos, Jr. A study of ELISA for schistosomiasis japonica using O-phenylenediamine, a substrate of peroxidase labeled antibody. *Jpn. J. Parasit.* 31:147-154, 1982 (text in Japanese)

Matsuda, H., H. Tanaka, S. Nogami and M. Muto. Mechanism of action of praziquantel on the eggs of *Schistosoma japonicum*. *Japan. J. Exp. Med.* 53:271-274, 1983

Matsuda, H., H. Tanaka, B.L. Blas, J.S. Noseñas, T. Tokawa, and S. Ohsawa. Evaluation of ELISA with ABTS, 2-2'-azino-di-(3-ethyl-benzthiazoline sulfonic acid), as the substrate of peroxidase and its application to the diagnosis of schistosomiasis. *Japan. J. Exp. Med.* 54:131-138, 1984

Matsuda, H., M. Nakao, M. Morita, and H. Tanaka. Sequential occurrence of IgM and IgG antibodies against egg and adult antigens in rabbits infected with *Schistosoma japonicum* observed by ELISA and changes after treatment with praziquantel. *Jpn. J. Parasit.* 33:163-170, 1984 (text in Japanese)

Mehlhorn, H., S. Kojima, H.J. Rim, P. Ruenwongsa, P. Andrews, H. Thomas, and B. Bunnag. Ultrastructural investigations on the effects of praziquantel on human trematodes from Asia: *Clonorchis sinensis*, *Metagonimus yokogawai*, *Opisthorchis viverrini*, *Paragonimus westermani*, and *Schistosoma japonicum*. *Arzneim.-Forsch.* 33(I):91-98, 1983

Miyasaka, K., H. Iida, N. Yoshioka, M. Miyasato, H. Tsutsumi, M. Itoh, H. Ishibashi, R. Kojo, K. Kamiki, Y. Sakata, and M. Schigematsu. The transition in the intradermal test for schistosomiasis japonica in the Chikugo River basin. *J. Kurume Med. Assn.* 44:319-334, 1981 (text in Japanese)

Miyasato, M., H. Iida, N. Yoshioka, H. Tsutsumi. Experimental pulmonary embolism with *Schistosoma japonicum* eggs. I. Histological observations on the tissue reaction to *S. japonicum* eggs and lycopodium powder. *Kurume Med. J.* 29:107-116, 1982

Miyasato, M. Experimental study of the influence of *Schistosoma japonicum* infection on carcinogenesis of mice liver treated with N-2-fluorenylacetamide (2-FAA). *Jap. J. Parasit.* 33:41-48, 1984

Miyasato, M., Y. Kakizoe, H. Iida, Y. Hirano, and H. Tsutsumi. Experimental pulmonary embolism with *Schistosoma japonicum* eggs. II. Pulmonary lesions in rabbits infected with *S. japonicum*. *Kurume Med. J.* 31:47-53, 1984

Mizuno, F., S. Nogami, M. Maruhashi, and T. Matsumura. Parasitic localization and growth in mongolian gerbil (*Meriones unguiculatus*) infected filarial *Dipetalonema viteae* under different lighting conditions. *Kobe J. Med. Sci.* 28:155-160, 1982

Mizuno, F., S. Nogami, M. Maruhashi, and T. Matsumura. Life cycle of African ticks, *Ornithodoros moubata* as a vector of filariae *Dipetalonema viteae* at the condition of continuous light and continuous darkness. *Japan. J. Trop. Med.* 10:219-227, 1982

Mizuno, F., S. Nogami, M. Maruhashi and T. Matsumura. Distribution of microfilariae intake from *Dipetalonema viteae* in *Meriones unguiculatus*. *Med. Biol.* 105:305-307, 1982 (text in Japanese)

Mori, A., T. Oda, and Y. Wada. Studies on the egg diapause and overwintering of *Aedes albopictus* in Nagasaki. *Trop. Med. (Nagasaki)* 23:79-90, 1981

Nagata, T. Evaluation of antigen preparations derived from *Schistosoma japonicum* eggs for enzyme-linked immunosorbent assay (ELISA). *Jpn. J. Parasit.* 31:303-312, 1982

Nakao, M., H. Matsuda, H. Tanaka, and T. Nagata. Comparison of three kinds of substrates for peroxidase-conjugated antibody in micro ELISA for schistosomiasis japonica. *Jpn. J. Parasit.* 30:197-204, 1981 (text in Japanese)

Nakao, M., H. Matsuda and H. Tanaka. Sandwich enzyme-linked immunosorbent assay (ELISA) for detection of circulating antigen in *Schistosoma japonicum* infections. *Jpn. J. Parasit.* 31:289-294, 1982

Nakamura, M., S. Nogami, Y. Hayashi, T. Shibuya, and H. Tanaka. A mass dissection method for collecting infective larvae of *Litomosoides carinii*. *Japan. J. Exp. Med. 54*:221–224, 1984

Narama, I., M. Tsuchitani, T. Umemura, and H. Kamiya. Pulmonary nodule caused by *Dirofilaria immitis* in a laboratory rabbit *(Oryctolagus cuniculus domesticus)*. *J. Parasit 68*:351–352, 1982

Nihei, N. On several methods to measure the size of particles composing mud skin around the egg of *Oncomelania nosophora*. *Ann. Ochanomizu Geogr. Soc. 21*:17–29, 1981 (text in Japanese)

Nihei, N., S. Asami, and H. Tanaka. Geographical factors influencing the population numbers and distribution of *Oncomelania nosophora* and the subsequent effect on the control of schistosomiasis japonica in Japan. *Soc. Sci. Med 15D*:149–157, 1981

Nihei, N. Medical geographical approach to schistosomiasis japonica. *Ann. Ochanomizu Geogr. Soc. 25*:24–27, 1984 (text in Japanese)

Noda, K., T. Shibuya, T. Kurata, Y. Hayashi, H. Matsuda, and S. Nogami. Transfer of delayed hypersensitivity induced by *Schistosoma japonicum* adult worm antigen in guinea pigs. *Japan. J. Exp. Med. 53*:173–176, 1983

Nogami, S., H. Tanaka, and M. Tomisato. Isolation of microfilariae using agarose gel. *Jpn. J. Parasitol. 31*:47–54, 1982 (text in Japanese)

Nogami, S., H. Tanaka, and H. Matsuda. Antimicrofilarial effect of diethylcarbamazine on microfilariae of *Litomosoides carinii* in the nude mouse. *Jpn. J. Parasitol. 31*:415–422, 1982 (text in Japanese)

Nogami, S., H. Tanaka, and H. Matsuda. Migration of adult worms of *Dipetalonema viteae* subcutaneously transplanted into the jird. *Jpn. J. Parasitol. 32*:137–142, 1983 (text in Japanese)

Nogami, S., M. Nakanura, and H. Tanaka. Low responsiveness of *Dipetalonema viteae* in the jird to filaricides. *Jpn. J Parasitol. 33*:141–147, 1984

Nojima, H., S. Noda, and A. Sato. Serial implantations of larval *Schistosoma mansoni* from infected to uninfected snails. *J Parasitol. 66*:478–482, 1980

Nojima, H., A.T. Santos, B.L. Blas, and H. Kamiya. The emergence of *Schistosoma japonicum* cercariae from *Oncomelania quadrasi*. *J. Parasitol. 66*:1010–1013, 1980

Nojima, H. and A. Sato. Effect of temperature and atmospheric pressure on the eggs of the intermediate snail hosts of *Schistosoma mansoni* and *S. haematobium*. *Jpn. J. Parasit 30*:121–126, 1981

Nojima, H., A. Sato, and K. Matsunaga. The emergence of schistosome cercariae from snails. 3. Combined effect of light and temperature on the emergence of *Schistosoma mansoni* and *S. haematobium*. *Jpn. J. Parasit. 30*:405–415, 1981

Nojima, H. and A. Sato. *Schistosoma mansoni* and *Schistosoma haematobium*: emergence of *schistosome cercariae* from snails with darkness and illumination. *Exp. Parasitol.* 189–198, 1982

Nojima, H., K. Matsunaga, A. Sato, and D.K. Koech. Relation between lunchtime and hourly output pattern of *Schistosoma haematobium* eggs in urine. *Jpn. J Parasit. 33*:297–303, 1984

Nonaka, S., Y. Hashiguchi, M. Kawabata, Y. Aoki, I. Tada, H. Figueroa M. and G. Zea F. Dermatological survey of onchocerciasis in Guatemala. *J. Dermatol. 7*:61–70, 1980

Nonaka, S., Y. Hashiguchi, K. Kawabata, T. Yoshimura, G. Zea F., and H. Figueroa M. Dermatological survey of onchocerciasis in Guatemala. *Proceedings of the Guatemala-Japan Joint Conference on Onchocerciasis Research and Control.* 174–175, 1982

Nonaka, S., T. Yoshimura, T. Sakamoto, and G. Zea F. Dermatological survey of onchocerciasis in Guatemala. II. Relationship between the prevalence rate of cutaneous changes and that of onchocerciasis. *J. Dermatol. 10*:439–445, 1983

Oda, T. and Y. Wada. Exsheathement and migration of microfilariae of *Brugia malayi (Che-ju strain) in mosquitoes*. *Trop. Med. (Nagasaki)* 22:27–33, 1980

Ogata, K. Preliminary report of Japan-Guatemala onchocerciasis control pilot project. Blackflies — The future for biological methods in integrated control — 105–115, Academic Press, London, 1981

Ogura, N., M. Kobayashi and H. Yamamoto. Studies on filariasis IV: Critical period of molting from the second to the third stage in filarial larvae of *Brugia pahangi Dokkyo*. *J. Med. Sci. 8*:74–81, 1981

Ohgo, T. Basic studies on the *Dirofilaria immitis* and the experimental infection with larval filariae in mice. *The Journal of the Osaka City Medical Center 29*:745–764, 1980 (text in Japanese)

Ohta, N., R. Hayase, T. Sasazuki, M. Hayashi, L.C. Tormis, B.L. Blas, and J.S. Nosenas. Association between HLA and clinical manifestation of schistosomiasis japonica. *Jpn. J Human Genet. 25*:159, 1980

Ohta, N., T. Sasazuki, and M. Iuchi. Immunogenetic analysis of patients with post-schistosomal liver cirrhosis in man. *Proc. Jap. Soc. Immunol. 11*:185–186, 1981 (text in Japanese)

Ohta, N., Y.K. Nishimura, M. Iuchi, and T. Sasazuki. Immunogenetic analysis of patients with post-schistosomal liver cirrhosis in man. *Clinical and Experimental Immunology 49*:493–499, 1982

Ohta, N., M. Minai, and T. Sasazuki. Antigen-specific suppressor T lymphocytes (Leu-2a^{+}3a^{-}) in human schistosomiasis japonica. *J. Immunol. 131*:2524-2528, 1983.

Oikawa, Y., T. Ikeda, and K. Fujita. IgE antibody response in rats infected with *Litomosoides carinii. Trop. Med.* 23:149-157, 1981.

Owhashi, M., and A. Ishii. Fractionation and characterization of allergens extracted from eggs of *Schistosoma japonicum. Int. Arch. Allergy appl. Immunol. 64*:146-156, 1981.

Owhashi, M., A. Ishii. Purification and characterization of a high molecular weight eosinophil chemotactic factor from *Schistosoma japonicum* eggs. *J. Immunol. 129*:2226-2231, 1982.

Owhashi, M., Y. Horii, and A. Ishii. Eosinophil chemotactic factor in schistosome eggs: A comparative study of eosinophil chemotactic factors in the eggs of *Schistosoma japonicum* and *S. mansoni in vitro. Am. J. Trop. Med. Hyg. 32*:359-366, 1983.

Pal, R., J.B. Kitzmiller, T. Kanda. Cytogenetics and genetics of vectors, proceedings of a symposium in the 16th International Congress of Entomology. Kodansha-Elsevier, 270, 1981.

Sadanaga, A., Y. Hayashi, H. Tanaka, S. Nogami, and A. Shirasaka. Evaluation of the transplantation method of adult *Brugia malayi* into the jird, *Meriones unguiculatus*, for testing macrofilaricides. *Japan. J. Exp. Med. 54*: 1984.

Sakaguchi, Y., S. Kihara, and I. Tada. The chromosomes and gametogenesis of *Dirofilaria immitis. Jap. J. Parasitol.* 29:377-381, 1980.

Sakaguchi, Y., I. Tada, L.R. Ash, and Y. Aoki. Chromosomes of two species of filarial worms, *Brugia pahangi* and *Brugia malayi* (Filariidae: Nematoda). *Chromosome Inf. Service 32*:12-14, 1982.

Sakaguchi, Y., I. Tada, L.R. Ash, and Y. Aoki. Karyotypes of *Brugia pahangi* and *Brugia malayi* (Nematoda: Filarioidea). *J. Parasitol. 69*:1090-1093, 1983.

Sakamoto, M. Changes of lymphatic system in cats experimentally infected with *Brugia* filariasis. *Tropical Medicine* 22:223-236, 1980 (text in Japanese).

Sakamoto, M., Y. Aoki, and Y. Nakajima. Experimental transmission of *Brugia pahangi* into mice. *Tropical Medicine* 24:17-25, 1982 (text in Japanese).

Sakamoto, M., Y. Aoki, M. Shimada, and Y. Nakajima. Experimental infection of rat with *Brugia pahangi. Tropical Medicine 24*:125-130, 1982.

Sakamoto, M., and G.Z. Flores. The change of blood picture of patients with onchocerciasis following administration of diethylcarbamazine. *Tropical Medicine* 25:47-50, 1983.

Sakamoto, M., E. Kimura, Y. Aoki, and Y. Nakajima. Subcutaneous and intraperitoneal inoculation of *Dirofilaria immitis* microfilariae into mice. *Jpn. J. Parasitol. 33*: in press.

Sano, M., M. Terada, A.I. Ishii, H. Kino, and M. Anantaphruti. Studies on chemotherapy of parasitic helminths (V). Effects of niclosamide on the motility of various parasitic helminths. *Experientia 38*:547-549, 1982.

Sasa, M., and T. Kurihara. The use of Poeciliid fish in the control of mosquitoes, in "Biocontrol of Medical and Veterinary Pests", Praeger, 36-53, 1981.

Sasazuki, T., N. Ohta, R. Kaneoka, and S. Kojima. Association between an HLA haplotype and low responsiveness to schistosomal worm antigen in man. *J. Exp. Med. 152*: 314s-318s, 1980.

Sasazuki, T., N. Ohta, and M. Iuchi. Schistosomiasis. *Proceedings of the 2nd Asia and Oceania Histocompatibility Workshop Conference*, eds. Simons, M.J. & B.D. Tait, pp. 402-406, 1981.

Sasazuki, T. HLA: Genetic control of immune response and disease susceptibility. *Jpn. J. Human Genet.* 27:81-94, 1982a.

Sasazuki, T. HLA-linked immune suppression genes and its role in the pathogenesis of immunological disorders. *Genetic aspect of immune response — Proceedings of the 6th Aso Symposium 1982*, pp. 50-58, 1982, eds. Onoue, K., T. Watanabe, and R. Mori, Nanzando, Tokyo, 1982b.

Sasazuki, T., Y. Nishimura, M. Mutoh, and N. Ohta. HLA-linked genes controlling the immune response and disease susceptibility. *Immunol. Rev. 70*:51-75, 1983.

Sato, K. and M. Suzuki. Radioallergosorbent Test for Human Filariasis Using Purified *Dirofilaria immitis* Antigen (FST). *Jap. J. Parasit. 30*:533-539, 1981.

Sato, K., T. Vijayamma, and M. Suzuki. Studies on Immunodiagnosis for Filariasis: Enzyme Immuno Assay Using Skin Test Antigen FST3 for Detecting IgE Antibody. *The Annual Reports of College of Medical Care and Technology*, 2:117-125, 1981.

Sato, K., M. Suzuki, and T. Vijayamma. Enzyme Immunoassay for Human Filariasis Using Purified *Dirofilaria immitis* Antigen (FST). *The Third Japan-Brazil Symposium on Science and Technology, Proceedings of Symposium*: 288-289, 1982.

Sato, K. and M. Suzuki. Studies on an Acid Protease from *Dirofilaria immitis. Jap. J. Parasit. 32*:577-586, 1983.

Sato, K., A. Suzuki, E. Takahashi, and Y. Tanaka. Purification and Characterization of an Acid Protease from *Dirofilaria immitis. The Annual Reports of College of Medical Care and Technology 5*: 1984 (in press).

Shibuya, T., B.D. Cabrera, H. Tanaka, F.S. Valeza, and R. Instrella. Comparison of the blood film, Millipore filter, and Nuclepore filter techniques for the detection of microfilaremia in a field survey in the Philippines. *Japan. J. Exp. Med.* 50:463-468, 1980.

BEST AVAILABLE COPY

Shibuya, T., H. Tanaka, B.D. Cabrera, F.S. Valeza, R. Instrella, T. Go, and S. Kobayashi. Low density microfilaremia of *Wuchereria bancrofti* infections in pre- and post-treatment phases in the Philippines. *Japan. J. Exp. Med.* 51:133–135, 1981.

Shibuya, T., H. Tanaka, Y. Oikawa, J. Kawashima, A. Sadanaga, and Y. Hayashi. Comparison of activity of diethylcarbamazine against *Litomosoides carinii* in Jms and Tum colonies of jirds, *Meriones unguiculatus*. *Japan. J. Exp. Med.* 51:223–227, 1981.

Shibuya, T., Y. Oikawa, J. Kawashima, and H. Tanaka. Treatment of cotton rat filariasis by spaced dosage of diethylcarbamazine. *Japan. J. Exp. Med.* 53:211–213, 1983.

Shigeno, S., E. Kimura, M. Sakamoto, and Y. Aoki. Effect of diethylcarbamazine on third and fourth stage of larvae of *Brugia pahangi* in Mongolian jird. *Japan. J. Parasit.* 32:465–473, 1983 (text in Japanese).

Shigeno, S., H. Yamashita, H. Takahashi, E. Kimura, Y. Aoki, and Y. Nakajima. Studies on *Brugia pahangi* in inbred hamsters. I. Susceptibility of inbred GN and APG hamsters. *Southeast Asian J. Trop. Med. Pub. Hlth.* 14:407–412, 1983.

Shimada, M. On the diurnal fluctuation in *Schistosoma haematobium* egg output. *Tropical Medicine* 22:237–244, 1980 (text in Japanese).

Suenaga, O., T. Nishioka, and T. Itoh. On the transition of canine heartworm prevalence among dogs in Nagasaki City, Western Japan. 1. The filarial prevalence among dogs in the city. *Trop. Med.* 22:35–45, 1980 (text in Japanese).

Suenaga, O. Cytoplasmic incompatibility and the role of *Wolbachia pipientis* in *Culex pipiens* complex. *Akaieka Newsletter* 5:16–29, 1981 (text in Japanese).

Suenaga, O. Treatment of *Wolbachia pipientis* infection with Tetracycline Hydrochloride and the change of compatibility in a strain of *Culex pipiens* complex. *Trop. Med.* 24:9–15, 1982 (text in Japanese).

Suenaga, O. Cytoplasmic incompatibility in a natural population of *Culex pipiens* complex in Nagasaki, Japan. *Trop. Med.* 24:79–85, 1982.

Suenaga, O., W. Liu, J. Miao, and W. Xu. Seasonal change in the oviposition activity of *Culex pipiens pallens* in Shanghai, China. *Trop. Med.* 24:229–234, 1982.

Suenaga, O., W. Liu, J. Miao, and W. Xu. Seasonal change in the oviposition activity of *Culex pipiens pallens* Coq. in Shanghai, China. *Contr. Shanghai Inst. Entomol.* 1982–1983, 29–33, 1983 (text in Chinese).

Suzuki, M. Malaria and Schistosomiasis. In "Medical Care and Prevention in the Tropics," 114–136, Fujitechnosystem, Tokyo 1982 (text in Japanese).

Suzuki, M. Parasitic diseases transmitted by insects (filariasis). In "Medical Care and Prevention in the Tropics," 145–148, Fujitechnosystem, Tokyo 1982 (text in Japanese).

Tada, I., T. Mimori, S. Nonaka, H. Figueroa, and M. Mazzotti. Clinical and histological observation of onchocerciasis cases tested in Guatemala. *Jap. J. Parasitol.* 30:501–507, 1981.

Tada, I., T. Mimori, Y. Sakaguchi, M. Kusano, Y. Hashiguchi, and M. Recinos C. The use of aceto-orcein stained squash preparations for enumeration of nuclei in microfilariae of various filarial parasites. *Amer. J. Trop. Med. Hyg.* 30:593–597, 1981.

Tada, I. and Y. Aoki. The laboratory diagnosis of onchocerciasis. *Afr. J. Clin. Exptl. Immunol.* 2:205–210.

Tada, I., Y. Aoki, Y. Hashiguchi, T. Yoshimura, Y. Ito, T. Ikeda, M. Kawabata, M. Takaoka, M. Sakamoto, K. Kondo, J.J.C. Orellana, B.H.A. Godoy, F.G. Zea, and C.M.M. Recinos. Epidemiological findings in San Vicente Pacaya, pilot area, Guatemala. *Proceedings of the Guatemala-Japan Joint Conference on Onchocerciasis Research and Control.* 122–124, 1982.

Tada, I., Y. Otsuji, R. Harada, T. Mimori, and H. Fukumoto. Skin test study of Bancroftian filariasis in Kuroshima Island, Okinawa. A 13 year longitudinal study during a control campaign. *Amer. J. Trop. Med. Hyg.* 31:962–967, 1982.

Tada, I. A comparative study on onchocerciasis between South and Central Americas. *Report of Overseas Scientific Research Team.* Ministry of Education, Science and Culture (No. 57041041), 1983.

Tada, I. Caracteristicas epidemiologicas y parasitologicas de laoncocercosis en Guatemala. Las filarias humanas en el Territorio Federal Amazonas (Venezuela). 151–154. PROICET AMAZONAS. 1983.

Tada, I., M. Korenaga, T. Mimori, M. Skamoto, T. Yoshimura, M.M. Recinos C., O.F. Flores, A. Lujan T., J.C. Castro, and G. Zea F. A comparative study of several diagnostic measures applied in Guatemalan onchocerciasis. *Jpn. J. Parasitol.* 34: (in press), 1985.

Tadano, T. Genetics of white eye in the mosquito *Aedes togoi*. *Ann. Trop. Med. Parasitol.* 4:647–654, 1980.

Tadano, T. Gynandromorphs of *Aedes togoi*. *Jap. J. Sanit. Zool.* 32:91–94, 1981.

Tadano, T. Linkage studies on an esterase locus in the mosquito *Aedes togoi*. *Biochem. Genet.* 20:711–721, 1982.

Tadano, T. Short antenna, a new mutant in the mosquito *Aedes togoi*. *Jpn. J. Sanit. Zool.* 33:105–109, 1982.

Tadano, T. Linkage studies on a new mutant, bleached pupa in *Aedes (Finlaya) togoi*. *Mosquito News* 42:61–65, 1982.

Tadano, T. A sex-linked mutant, hooked leg, in the mosquito *Aedes (Finlaya) togoi*. *Mosquito News* 42:72–75, 1982.

Tadano, T. Genetic linkage relationships between two enzyme loci, *Est-2* and *Acph*, in the mosquito *Aedes (Finlaya) togoi*. *Biochem. Genet. 21*:477–485, 1983.

Tadano, T. Genetics of octanol and glycerophosphate dehydrogenases in the mosquito *Aedes (Finlaya) togoi*. *Biochem. Genet. 21*:1167–1174, 1983.

Tadano, T. A genetic linkage map of the mosquito *Aedes togoi*. *Jpn. J. Genet. 59*:165–176, 1984.

Tagawa, M., K. Kurokawa and H. Tanaka. Analysis of immunoelectrophoretic patterns on dogs artificially infected with *Dirofilaria immitis*. *Bull. Nippon Vet. Zootech. College 29*:14–22, 1980 (text in Japanese).

Tagawa, M., K. Uematsu, K. Kurokawa and H. Tanaka. Circulating antigens and antibodies of *Dirofilaria immitis* in the dog detected by counterimmunoelectrophoresis. *Japan. J. Vet. Sci. 45*:323–329, 1983.

Tajima, K., K. Fujita, S. Tsukidate, T. Oda, S. Tominaga, T. Suchi, and Y. Hinuma. Seroepidemiological studies on the effects of filarial parasites on infestation of adult T-cell leukemia virus in the Goto Island, Japan. *Gann 74*:188–191, 1983.

Takai, K., T. Kanda, W.H. Cheong, and K.P. Loong. A recessive mutant, *pale brown eye*, and a cytoplasmic deleterious effect on viability in *Anopheles leucosphyrus* (Diptera:Culicidae) *J. Med. Entomol.* 21, 145–148, 1984.

Takai, K., T. Kanda, Y. Oguma, W.H. Cheong, A.M. Joesoef, and S. Sucharit. Postmating reproductive isolation between 7 members of the *Anopheles hyrcanus species group in East Asia. Jap. J. Sanit. Zool. 35*:251–259, 1984.

Takaoka, H. Seasonal occurrence of *Simulium ochraceum*, the principal vector of *Onchocerca volvulus* in the southeastern endemic area of Guatemala. *Am. J. Trop. Med. Hyg. 30*:1121–1132, 1981.

Takaoka, H., K.M. Hansen, H. Takahasi, J.O. Ochoa, and E.L. Juarez. Development of *Onchocerca volvulus* larvae in *Simulium ochraceum* at various altitudes in Guatemala with special reference to the ambient temperature. *Jpn. J. Trop. Med. Hyg.* 9:187–197, 1981.

Takaoka, H. Review on the biology and ecology of the adult blackflies in relation to the transmission of onchocerciasis in Guatemala. *Jpn. J. Trop. Med. Hyg. 10*:1–12, 1982 (in Japanese).

Takaoka, H. Observations on the bionomics of larval and man-biting female population of *Simulium horacioi*, a new potential vector of *Onchocerca volvulus* in Guatemala. *Jpn. J. Trop. Med. Hyg. 10*:49–62, 1982.

Takaoka, H., J.O. Ochoa, E.L. Juarez, and K.M. Hansen. Effect of temperature on development of *Onchocerca volvulus* in *Simulium ochraceum*, and longevity of the simuliid vector. *J. Parasitol.* 68:478–483, 1982.

Takaoka, H. Chapter VI, "Entomology." 36–65 pp. In: Tada, I. (ed.) *A comparative study on onchocerciasis between South and Central Americas*. 86 pp. Kumamoto, Japan, 1983.

Takaoka, M., A.T. Lujan, Y. Hashiguchi, M. Kawabata, Y. Ito, and S. Hayashi. Evaluation of the double diffusion test for serodiagnosis of onchocerciasis in Guatemala. *Jap. J. Parasit. 32*:451–457, 1983.

Takaoka, H., H. Suzuki, S. Noda, J.O. Ochoe A., and I. Tada. The intake, migration, and development of *Onchocerca volvulus* microfilariae in *Simulium haematopotum* in Guatemala. *Jpn. J. Sanit. Zool. 35*:121–127, 1984.

Takaoka, H., H. Suzuki, S. Noda, I. Tada, M.G. Básañez, and L. Yarzába. Development of *Onchocerca volvulus* larvae in *Simulium pintoi* in the Amazonas region of *Venezuela. Am. J. Trop. Med. Hyg. 33*:414–419, 1984.

Tanabe, M., S. Kobayashi, S. Miura, T. Takeuchi, Y. Kaneda, and K. Asami. A globin specific protease in *Schistosoma mansoni* I. Cooperation with a protease in mouse serum. *Jpn. J. Parasit.* 29:195–201, 1980.

Tanabe, M., M. Masuda, T. Takeuchi, S. Kobayashi, and K. Asami. Biochemical analysis of granuloma formation around *Schistosoma mansonigges* (I). *Jpn. J. Parasit.* 29:50, 1980, suppl.

Tanabe, M., and K. Asami. Patho-biochemical changes in spleen and mesenteric lymph node in murine schistosomiasis mansoni. *Jpn. J. Parasit. 30*:44, 1981, suppl.

Tanabe, M., S. Miura, S. Kobayashi, and K. Asami. Changes in activities of lysosomal enzymes in experimental schistosomiasis mansoni. *Jpn. J. Parasit. 32*:271–278, 1983.

Tanabe, M., and K. Asami. Biochemical changes in the fibrotic liver of experimental murine schistosomiasis mansoni. *Jpn. J. Parasit. 33*:43, 1984, suppl.

Tanaka, H., H. Nakai, K. Omoto, T. Shibuya, M. Hirai, A.S. Mercado, I.G. Pagaran, and W.S. Eviota. The high prevalence of *Wuchereria bancrofti* infections in indigenous tribes in northern Mindanao, Philippines. *Japan. J. Exp. Med. 50*:85–89, 1980.

Tanaka, H. and T. Shibuya. Modification of Nuclepore filtration technique for microfilaria detection. *Japan. J. Exp. Med. 50*:393–394, 1980.

Tanaka, H. Periodicity of microfilariae of human filariasis analyzed by a trigonometric method (Aikat and Das). *Japan. J. Exp. Med 51*:97–103, 1981.

Tanaka, H., M. Takaoka, H. Matsuda, and Y. Hayashi. Effect of diethylcarbamazine on microfilariae of *Brugia malavi* in the jird, *Meriones unguiculatus*. *Japan. J. Exp. Med. 51*:195–196, 1981.

Tanaka, H., B.L. Blas, J.S. Nosenas, H. Matsuda, M. Ishige, H. Kamiya, I. Murata, Y. Hayashi, and A.T. Santos, Jr.

Longitudinal study on *Schistosoma japonicum* infections in the Philippines. 3. Incidence and environmental modification at Dagami, Leyte. *Japan. J. Exp. Med. 53*:87-94, 1983.

Tanaka, H., H. Matsuda, B.L. Blas, J.S. Noseñas, M. Hayashi, M. Iuchi, M. Nakao, and A.T. Santos, Jr. Evaluation of micro-ELISA for schistosomiasis japonica using crude egg antigen. *Japan. J. Exp. Med. 53*:147-154, 1983.

Tanaka, I., Y. Hashiguchi, T. Okazawa, J.O. Ochoa A., and I. Tada. Duration of blood feeding of *Simulium ochraceum* in relation to intake of *Onchocerca volvulus* microfilariae. *Jap. J. Sanit. Zool. 31*:209-214, 1980.

Terada, M., M. Anantaphruti, A.I. Ishii, H. Kino, and M. Sano. Studies on Chemotherapy of Parasitic Helminths (X). *In Vitro* Effects of Known Antischistosomal and Neuropharmacological Drugs on the Motor Activity of Adult *Schistosoma Japonicum*. *Jap. J. Parasit. 31*:313-320, 1982.

Terada, M., A.I. Ishii, H. Kino, Y. Fujiu, and M. Sano. Studies on chemotherapy of parasitic helminths (IX). Effects of praziquantel on the motility of various parasitic helminths and isolated host tissues. *Experientia 38*:549-553, 1982.

Terada, M., M. Sano, A.I. Ishii, H. Kino, S. Fukushima, and T. Noro. Studies on chemotherapy of parasitic helminths (III). Effects of tuberostemonine from *Stemona japonica* on the motility of parasitic helminths and isolated host tissues. *Folia pharmacol. japon. 79*:93-103, 1982.

Terada, M., M. Sano, A.I. Ishii, H. Kino, S. Fukushima, and T. Noro. Studies on chemotherapy of parasitic helminths (IV). Effects of alkaloids from *Sophora flavescens* on the motility of parasitic helminths and isolated host tissues. *Folia pharmacol. japon. 79*:105-111, 1982.

Tomisato, N., Y. Nakajima, H. Nariuchi, and S. Nogami. Detection of IgG antibodies in mongolian jirds infected with *Brugia pahangi* by enzyme-linked immunosorbent assay. *Jpn. J. Parasitol. 32*:43-54, 1983.

Tongu, Y., J.S. Lai, T. Aji, S. Suguri, H.L. Shing, and S. Inatomi. The incidence of abnormal spermatozoa in *Armigeres subalbatus* (Coquillett, 1898). *Jpn. J. Sanit. Zool. 33*:123-127, 1982.

Tsuruoka, H., Y. Chigusa, N. Ogura, M. Kobayashi, and H. Yamamoto (1983): An application of the thin layer gel-electrophoresis for the analysis of esterase isozyme in the mosquitoes, *Aedes aegypti*. Basic study on the condition of electrophoresis. *Dokkyo J. Med. Sci. 10*:101-112.

Uni, S., I. Kimata, and S. Takada. Cross-section morphology of *Dirofilaria ursi* in comparison with *D. immitis*. *Jap. J. Parasit. 29*:489-497, 1980.

Uni, S. Filarial parasites from the black bear of Japan. *Ann. Parasitol. Hum. Comp. 58*:71-84, 1983.

Usawattanakul, W., T. Kamijo, and S. Kojima. Comparison of recovery of schistosomula of *Schistosoma japonicum* from lungs of mice and rats. *J. Parasit. 68*:783-790, 1982.

Wada, Y. Biting midges and their control. *Seikatsu to Kankyo 25*:29-35, 1980 (text in Japanese).

Wada, Y. About the origin of filariasis. *Kankyo Eisei 28*:6-11, 1981 (text in Japanese).

Wada, Y. Species of *Culicoides* collected from Fukue Island, Goto Islands. *In*: Natural History of Goto Islands, p. 339, 1981 (text in Japanese).

Wada, Y. Species of *Culicoides* in Danjo Islands (Diptera: Ceratopogonidae). *In*: Natural History of Goto Islands, p. 699, 1981 (text in Japanese).

Wada, Y. Transmission dynamics of onchocerciasis by *S. ochraceum* in the pilot control area in Guatemala. *Proceedings of the Guatemala-Japan Joint Conference on Onchocerciasis Research and Control*:102-103, 1981.

Wada, Y. Theoretical approach to the epidemiology of Onchocerciasis in Guatemala. *Jpn. J. Med. Sci. Biol. 35*:183-196, 1982.

Wada, Y., M. Takahashi, and T. Ikemoto. Mosquitoes of the Institute for Nature Study (Insecta: Diptera). *Misc. Rep. Inst. Nature Study No. 15*:1-2, 1984 (text in Japanese).

White, G.B. and T. Kurihara. Host plant adaptations of the filariasis vector mosquito *Aedes poicilius*. *Trans. Roy. Soc. Trop. Med. Hyg. 74*:683, 1980.

Yamamoto, H., N. Ogura, M. Kobayashi, and Y. Chigusa. Studies on filariasis II: Exsheathement of the microfilariae of *Brugia pahangi* in *Armigeres subalbatus*. *Jpn. J. Parasit. 32*:287-292, 1983.

Yamashita, S., S. Shigeno, E. Kimura, and Y. Aoki. The effect of diethylcarbamazine on microfilariae of *Brugia pahangi* in Mongolian jirds *(Meriones unguiculatus)*. *Tropical Medicine 25*:113-117, 1983.

Yanagawa, T., F. Kasagi, and T. Yoshimura. A method to estimate incidence rates of onchocerciasis with consideration of false negative due to skin snip biopsy. *Biometrics. 40*:301-311, 1984.

Yasukawa, M., and T. Sasazuki. Genetic control of immune response in man. *Men-eki-gaku 3*:137-147, 1982. Eds. Kishimoto, T. and T. Hirano, Nakayama-shoten, Tokyo (text in Japanese).

Yasuraoka, K. Biological Agents and Geographic Pathology. *In*: Geographic Pathology of Diseases (Ed. S. Yamaguchi), Asakura Shoten, Tokyo, Japan, 135-155, 1980 (text in Japanese).

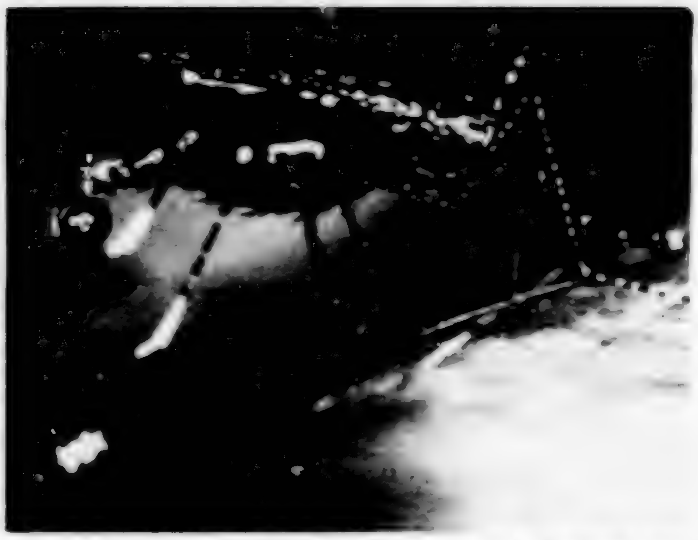
Yasuraoka, K., H. Tanaka, A.T. Santos, Jr., E.J. Zerrudo, B.L. Blas and J.S. Nosenas. *Proceedings of the Philippine-Japan Joint Conference on Schistosomiasis Research and Control*. Japan Inter. Coopera. Agen. Tokyo, 123 pp., 1980.

Yasuraoka, K. Schistosomiasis as a Parasitic Zoonosis. *In*: Parasitic Zoonoses in Japan (Ed. S. Hayashi et al.), Buneido, Tokyo, Japan. pp. 131–142. 1983 (text in Japanese).

Yoshimura, T., Y. Hashiguchi, K. Kawabata, O.F., C. Flores, O.O. Gudiel, and L. Mazariegos, E.C. Prevalence and incidence of onchocerciasis as baseline data for evaluation of vector control in San Vicente Pacaya, Guatemala. *Trans. R. Soc. Trop. Med. Hyg. 76(1)*:48–53, 1982.

Yoshimura, T., E.C. Mazariegos, O.O. Gudiel P., and G. Zea. F. Data recording and analysis system for the epidemiological surveys in Guatemala. *Proceedings of The Guatemala-Japan Joint Conference on Onchocerciasis Research and Control.* 125–126. 1982.

Zea, F., G., Y. Hashiguchi, M. Kawabata, I. Tada, T. Yoshimura, C.O. Flores, and M.M. Recinos. Guatemalan Onchocerciasis: Skin Snipping Methods and Microfilarial Densities in a Given Minute Area of the Skin. *Japan J. Trop. Med. Hyg. 8(1)*:23–32. 1980.



***Anopheles dirus*, the primary vector of malaria in Southeast Asia and southern China, taking a blood meal.**

U.S. Parasitic Diseases Panel

BROAD GOALS

Since the inception of the U.S.-Japan Cooperative Medical Science Program in 1965, the focus of the Joint Panels on Parasitic Diseases was restricted to the two important disease entities of schistosomiasis and filariasis. The current status of these two groups of parasites as major causes of human suffering throughout the world's tropical and subtropical regions is generally little improved. In fact, the diseases are still spreading in many developing countries. It is generally estimated that each of these diseases afflicts more than 250 million people. Although direct mortality from these infections is low, their profound effect upon human health and well-being and their interaction with malnutrition and other parasites and microbial agents contributes to substantial morbidity and places a significant burden on health services in developing countries where they occur. The importance of these two diseases was underscored when, in 1976, the World Health Organization included schistosomiasis and filariasis as two of the six diseases for selected study in their Special Programme for Research and Training in Tropical Diseases.

The original 1965 guidelines directed the Panels on Parasitic Diseases to focus their activities on the two important vector-borne human helminthiases, schistosomiasis, and filariasis. This rather limited focus was reaffirmed in 1975 when the guidelines were reviewed by the panels. A wide variety of studies, involving both basic and applied research on these two diseases, were continued. The success of this program over the two decades of the Cooperative Medical Science Program is clearly demonstrated by growth in research publications and the appearance of many new investigators who pursue relevant research.

However, by the early 1980s it became apparent that application of some of the newer research technologies in immunology and molecular biology, employed to study blood and tissue protozoan infections, such as malaria, leishmaniasis, and trypanosomiasis, had considerable relevance for research in schistosomiasis and filariasis. The panels noted the similar investigative problems between many helminthic and protozoal diseases including vector interactions, pathogenesis, immunologic consideration, and other factors. These objective discussions resulted in change of guidelines with inclusion of malaria and other vector-borne parasitic diseases. The new guidelines are as follows:

"The Parasitic Diseases Panels have a primary interest in research on selected helminthic and protozoal diseases with special emphasis on vector-borne parasitic diseases such as, but not limited to, malaria, schistosomiasis, and filariasis. Sponsored studies share a long-term goal of alleviation of these diseases. Strategies include vector control, interruption of the parasite life cycle, and reduction of the amount and effects of infection in man by vaccination, chemoprophylaxis, or other modalities. Both basic and applied work is supported in the following areas:

Epidemiology
Vector Biology and Control
Parasite Biology
Host-Parasite Interactions
Pathology
Immunology
Biochemistry
Chemotherapy

Special emphasis will be given to studies designed to explore the role of membrane chemistry and antigen differentiation in infection and immunity. In addition, the panel is concerned with supply of parasites and infected animals to investigators and with availability of research workers in these fields."

Appropriately, the focus of the Parasitic Diseases Panels is to promote, encourage, and abet cooperative international research on not only filariasis and schistosomiasis but malaria and other vector-borne parasitic diseases. The biologic nature of the parasites involved and their subsequent human effects presume a long-term commitment to develop an understanding of the disease processes and formulation of specific means for their control. Complexities of their life cycles involve molluscan and arthropod vectors, as well as human and other mammalian hosts. This necessitates a multivariate look at all facets of their life cycle in order to find the weak links which might be exploited by scientists who work in specific and related fields. Such disciplines as parasitology, malacology, entomology, genetics, ecology, biochemistry, pharmacology, immunology, molecular biology, and other basic disciplines must all interact, in the laboratory and in the field, to achieve success in combatting these parasites.

Significant advances were made since 1979 in schistosomiasis and filariasis research. This is vividly reflected in the large number of publications which are attributable to sponsorship of studies under the Program's aegis. This logarithmic growth involved investigators, and newly advanced knowledge relates directly to the guidelines' directive to supply parasites, infected animals, and vectors to investigators. The complex nature of the life cycles of schistosomes and filariae precludes some scientists who lack facilities for maintenance of these cycles from working on these organisms. Many of the important facets of basic investigations are handled by technical personnel with little or no parasitological expertise. They bring their own special expertise to help resolve some of the problems associated with these diseases.

In the following report brief summaries of each of the preceding three 5-year reports, 1965-1970, 1970-1975, and 1975-1980, are provided. These are followed by a more detailed summary of the notable developments of the last 5 years of research in the fields of schistosomiasis and filariasis.

SHORT SUMMARY OF THE FIRST FIVE YEARS

A broad range of investigation on filariasis and schistosomiasis included immunology, pathogenesis, pathophysiology, biochemistry, and chemotherapy. The following research accomplishments were highlighted on schistosomiasis: (1) demonstration of protective immunity in primates against *Schistosoma japonicum* by exposure to homologous, x-irradiated cercariae; (2) demonstration of the relationship between cell-mediated immunity and schistosome granuloma formation; (3) definition of the lipid metabolism of schistosomes, and of their dependence on an exogenous supply of cholesterol; (4) elaboration of the conformational requirements for schistosomicidal activity of nitroheterocyclic drugs; and (5) establishment of new experimental models of *Schistosoma haematobium* infection in subhuman primates, with pathologic reactions similar to that of humans.

In filariasis research, a new small laboratory animal model of lymphatic filariasis was established through infection of the jird, *Meriones unguiculatus*, with *Brugia pahangi* and *B. malayi*. This model generated many new advances in our understanding of lymphatic filarial infections. Quantitative studies on pathogenesis of *Brugia* infections in cats were initiated. Efforts to improve filarial serodiagnosis were instructive in detection of extensive cross-reactivity between filarial and other helminth antigens. Field studies were reported on the vector mosquito of the *Aedes scutellaris* group in the Pacific as were biochemical studies on the bacteria-free rearing of *Aedes* mosquitoes which defined the host's requirements of RNA and of zinc.

During this period filariasis research was devoted principally to elaboration of model systems for the laboratory study of various filarial parasites. The classical, laboratory-maintained filariae—*Litomosoides carinii*, *Dipetalonema viteae*, and *Dirofilaria immitis* were utilized but increased progress was made in studies on the lymphatic-dwelling filariae of the genera *Brugia* and *Wuchereria*. It showed for the first time that the human species, *Wuchereria bancrofti*, matured, though microfilaremias did not develop, in macaque monkeys. Studies on the pathogenesis of *Brugia malayi* and *B. pahangi* infections in dogs and cats gave new insight into the underlying mechanisms of human pathologic changes caused by these lymphatic filariae.

The importance of the Mongolian jird, *Meriones unguiculatus*, as a laboratory host for the lymphatic filariae was confirmed and extended. A significant finding was that intraperitoneal inoculation of infective larvae resulted in development of adult worms and production of microfilariae in the body cavity. This facilitated recovery of all stages of the parasite for use in experimental procedures, the collection of material for use as antigen in serological and immunological studies, and development of biochemical studies on these parasites. The *Dirofilaria* dog model and the various *Brugia* models showed that separate immunological mechanisms were operative in the suppression of microfilaremia versus protection from reinfection.

Despite increased study, no new antifilarial drugs were shown to be superior to diethylcarbamazine. A significant new vector development was the demonstration that cytoplasmic incompatability in *Culex* related to the presence of a rickettsia-like organism, *Wolbachia pipientis*, which could have considerable significance in vector control.

SHORT SUMMARY OF THE SECOND FIVE YEARS

Research efforts were confined to schistosomiasis and filariasis; investigators working in schistosomiasis exceeded those in filariasis; the character of work was more sophisticated. The latter related specifically to the opportunity to employ defined laboratory animal models with shortened life cycles for schistosome parasites.

Major advances in schistosomiasis included the sequential monitoring of the immunopathology of cercaria-induced infections by modern quantitative immunological and pathophysiological techniques. The role of cell-mediated immunity in granuloma formation was best demonstrated by use of guinea pigs and chickens, which are normally unsusceptible to schistosomiasis. The immunological mechanisms activated by schistosome eggs vary among parasite species and granulomatous responses are subject to eliciting and modulating influences. This has considerable significance since modulation of the host cell response to schistosomes could have therapeutic value in human infection, particularly in the acute, severe, "toxemic" stage.

Problems associated with the mutagenic, teratogenic, and carcinogenic potential of antischistosomal compounds were significant. These led to numerous studies on analogs of effective compounds and development of drug resistance. Substantial progress in cultivation of schistosome larval stages was made; identification of the complete life cycle was not accomplished. A major development was the successful establishment of a cell line from *Biomphalaria glabrata* embryos.

SHORT SUMMARY OF THE THIRD FIVE YEARS

A wide variety of animal models was used for schistosome research with expanded use of inbred strains of mice and utilization of hamsters, rabbits, and primates. This led to significant advances and a better understanding of many features of schistosome infections which included mechanisms of protection and immunoregulation and the chronology of the pathologic response. In studies of *Schistosoma japonicum* the Philippine strain of the parasite was consistently more pathogenic than those of China, Taiwan, or Japan. The recently described *S. mekongi* is highly pathogenic for hamsters and mice but dogs, the natural reservoir, suffer only minor pathologic changes and tend to self cure over time. The baboon is the best animal host for study of pathologic changes due to *S. haematobium* infection.

Immunopathogenic studies showed the dependence of T lymphocytes and schistosome ova granuloma formation. There are differences in biologic and host immune responsiveness to infections with *S. mansoni* and *S. japonicum* which suggests possible differences in egg antigens between the species. *In vitro* systems were developed in tissue culture which showed the formation of actual granulomas around eggs.

Much work was related to the potential development of a schistosome vaccine. The purification and identification of antigens, particularly those at the egg stage, were notable advances. From these studies hybridoma technology was used for development of monoclonal anti-soluble egg antigen component antibodies. This led to development of a very sensitive radioimmunoassay procedure for the diagnosis of *S. mansoni* infection.

It was shown that eosinophils cause a small but significant antibody-dependent toxic effect on culture derived schistosomula. There is evidence that activated macrophages may play an important role in rejection of schistosomula and that macrophage activation may be a major determinant factor of the immune status of the host. Through use of various model systems it was shown that inbred mouse strains vary widely in their protection due either to chronic primary infection or irradiated-cercarial immunization.

The need for better antischistosomal drugs remained a high priority for research. Despite considerable progress in cultivation of schistosome life cycle stages there was a major gap in the inability to promote and maintain reproductive activity under axenic conditions. This has considerable relevance in development of antischistosomal drugs. Finally, the development of molluscan cell culture lines made new research opportunities possible such as evaluation of defense mechanisms in molluscs.

A major advance in filariasis research was production of experimental patent infections of *Wuchereria bancrofti* in Taiwan macaques. Microfilaremias were low and transient yet this accomplishment was the first definitive indication for the eventual establishment of a primate model for the study of classical *W. bancrofti* infection. This parasite is the major cause of human filarial elephantiasis. Jirds and other rodents were widely exploited for pathologic and immunologic studies. Concurrent feline infections with *Brugia malayi* and streptococci resulted in tissue inflammation and edema consistent with that of chronic human infections. In addition to the lymphatic filariae, it was demonstrated that the African eye worm, *Loa loa*, could be maintained routinely in baboons and several species of monkeys; deerflies from the southeastern United States can serve as efficient experimental vectors. Use of the Mongolian jird-*Brugia* model for study of the immune response in filariasis provided evidence for the role of cell-mediated immunity in these infections. Filarial infections induced suppressor cell activity which inhibited lymphocyte proliferation *in vitro*. Increased levels of circulating immune complexes were noted in the sera of jirds infected with *B. pahangi*, but there was no correlation between immune complex levels and the numbers of circulating microfilariae. Studies were pursued on the biochemistry of larval and adult filariae, with a view toward developing better chemotherapeutic agents. Pyruvate metabolism was evaluated in *B. pahangi* and *Dipetalonema viteae*. The tricarboxylic acid cycle pathway is regarded of doubtful importance as an energy yielding pathway in these parasites.

Through Panel-sponsored contract research short- and long-term cryopreservation of microfilariae and infective-stage filarial larvae of sheathed and unsheathed species was accomplished. This provided opportunities for the transport of rare or scarce materials to laboratories capable of working with various aspects of these parasites. Work with *W. bancrofti* and *Mansonella ozzardi* could be enhanced considerably. Studies on vector biology showed the usefulness of American species of deerflies for transmission of *Loa* infection and that local species of blackflies, *Simulium* spp., were not infected with *Onchocerca volvulus*. This was due to the presence of teeth in the mouthparts of these flies which damage ingested microfilariae.

PROGRESS IN PRIORITY AREAS

Schistosomiasis

Vector biology

Ongoing studies were directed to isolation and characterization of antigens shared between *Schistosoma mansoni* and its intermediate host, *Biomphalaria glabrata*. These antigens are being tested for their potential as immunodiagnostic reagents and as vaccines. Use of heterospecific and monospecific (monoclonal) antibody probes have revealed a high degree of antigenic similarity between schistosome larvae (miracidia and sporocysts) and the snail (serum and hemocytes).

Work has continued on evaluation of the geographic distribution and snail host-parasite relationships of Asian schistosomes which infect man and animals; these include *S. mekongi*, *S. japonicum*, and *S. malayensis*. The genus *Tricula* transmits *S. mekongi* and the closely related genus *Robertsiella* transmits the Malaysian schistosome, *S. malayensis*. In a collaborative study between American and Chinese scientists in Yunnan Province in China, it was shown that *Tricula* snails occur extensively in northern Burma, China, and into the Mekong River area. These findings have special relevance to understanding the ecological divergence of oncomelanid and triculid snails.

Parasite biology and biochemistry

Attention was focused on integumentary surface phenomena in male and female schistosomes. Female worms obtain glucose from their male partners and they draw upon the glycogen reserves of males. Thus, the male functions not only for reproduction but helps buffer the female from certain nutritional stresses by providing an optimal microenvironment for the mate. These data help explain why females are unable to develop and reach maturity in the absence of males.

A number of studies were directed to isolation and characterization of schistosome proteins and proteolytic enzymes. Proteolytic enzymes of *S. mansoni* eggs are being studied to determine their role in the movement of eggs through tissues and their stimulation of host immune responses and granuloma formation. A large number of hybridoma cell lines were cloned which produce monoclonal antibodies and recognize a wide variety of epitopes from *S. mansoni* and *S. japonicum* egg antigens. Testing of some monoclonals in enzyme-linked immunosorbent assays (ELISA) indicated the potential yield of highly specific and sensitive immunodiagnostic procedures. Other studies identified the species-specific and gender-specific proteins of *S. mansoni*, *S. japonicum*, and *S. haematobium*. The immunoreactivity of these proteins was characterized by use of sera of infected hosts and with monoclonal antibodies. These studies should help develop probes (purified schistosomal proteins and monoclonal antibodies) useful for identification of the antigen and antigen/antibody complexes associated with granulomas and glomerular nephritis in infected mice. Drug development for use against schistosomes is in progress. Pharmacologic differences between longitudinal and circular muscles of these parasites are being examined which may be useful as targets for antischistosomal drugs. The calcium regulatory protein, calmodulin, is important for schistosomes. Inhibition of its action by potential chemotherapeutic agents may suggest new agents for drug therapy.

Immunity, immunopathology, and immunoregulation

The complexity of cell interactions that take place during the chronic inflammatory process of schistosomiasis is under study by a number of investigators. In addition to the role of lymphocytes, macrophages and mast cells as well as lymphocytes may play an accessory regulatory role in the inflammatory and reparatory process in the schistosome egg granuloma. Development of schistosome egg antigen-specific T cell clones was a major advance in the continued evaluation of the immunopathogenesis and immunoregulation of *S. mansoni* egg-induced granulomas. These clones respond specifically to schistosome egg antigen by undergoing blastogenesis, by producing local delayed type hypersensitivity reactions, by producing various lymphokines, and initiating the generation of granulomas *in vitro*. The role of the granulomatous inflammation elicited by schistosome eggs in hepatic fibrogenesis is being examined. Findings provide strong circumstantial evidence that hepatic fibrosis in schistosomiasis, characterized by peripheral fibrosis, vascular smooth muscle hyperplasia, and endothelial proliferation results from biologically active molecules elaborated by cells in the granulomas. Not only are these studies relevant for schistosomiasis, but they provide a model which may explain the pathogenesis of tissue fibrosis in other diseases associated with chronic inflammation, including lymphatic filariasis

Several investigators are studying immunity against challenge infection by *S. mansoni* cercariae after initial infection or previous exposure to highly irradiated cercariae. With irradiated cercariae it appears that development of protection correlates with delayed type hypersensitivity to schistosomal antigens, but not with antigen-induced blastogenesis or anti-schistosomal antibody responses. In baboons, the use of irradiated cercariae results in a reduction in the size of hepatic and intestinal egg-focussed granulomas.

A field study with *S. japonicum* infection dealt with a long-term longitudinal seroepidemiological analysis of infection in elementary school children in several endemic areas on Leyte, the Philippines. The development of sensitive serodiagnostic procedures showed that diagnosis only by multiple stool examinations will considerably underestimate the true incidence of infection. These studies provided data showing no evidence from immunologic studies that implementation of an integrated control program resulted in reduction of transmission of schistosomiasis during the period under study

Filariasis

Advances made in filarial research in humans and animals since the advent of the U.S.-Japan Cooperative Medical Science Program have been enormous. Fifteen years ago, few investigators worked with experimental filariasis. The species of parasites under study were generally limited to such animal species as *Dirofilaria immitis*, *Litmosoides carinii* and initial work with *Brugia pahangi* in cats and primates. Development of a variety of animal models as hosts for human and zoonotic filariae served as an effective catalyst which has now extended the field to include work on immunology, pathology, and chemotherapy. Various rodents and the ferret are used for studying lymphatic filarial infections; various non-human primates are excellent hosts for *Wuchereria bancrofti* and species of the genera *Dipetalonema* and *Mansonella*. Although there is no suitable small laboratory animal host for *Onchocerca volvulus*, other species of *Onchocerca* which occur in domesticated animals are proving useful during the search for a suitable host for *O. volvulus*.

Investigations on schistosomiasis prospered because of the ready availability of immunologically well-defined laboratory animal hosts for these infections. Only recently have such laboratory animal hosts been developed for filarial studies. With the advent of these laboratory models and the entry of new investigators into the field, the prospects for great progress in the better understanding of filarial infections are excellent. Examples of recent findings which enhanced filarial research are the development of cryopreservation methodologies for microfilariae, larval stages, and adult worms. Various species of ***Brugia*** and ***Dirofilaria***, which possess individual characteristics and peculiarities, have been successfully cryopreserved for extended periods either as microfilariae or larval stages within the arthropod vectors. This contribution served directly from Panel-sponsored contract research.

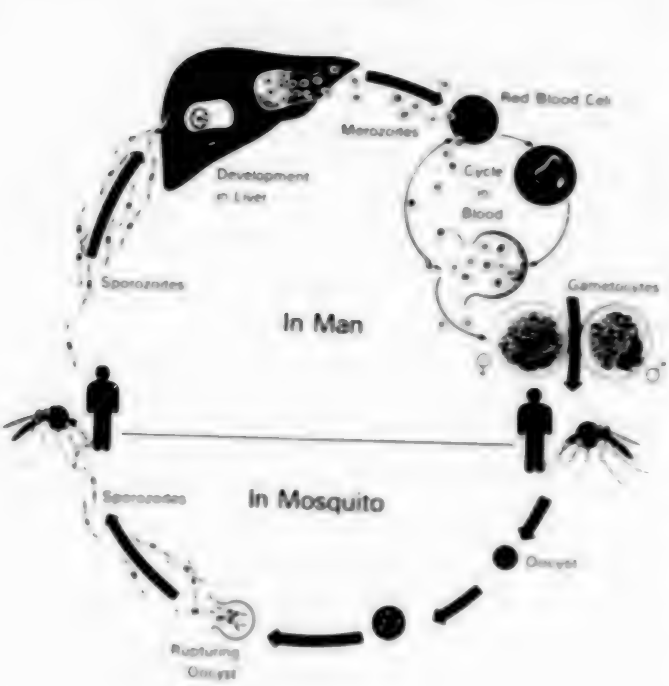
Vector and filarial biology

Vector studies in Guatemala, Panama, and Colombia showed that there is a potential hazard for extension of onchocerciasis and mansonellosis in new geographic areas due to exposure of greater numbers of persons through new road or land development projects. Fortunately, new rearing and maintenance techniques and renewed interest in vector-parasite interactions resulted in a resurgence of vector studies in filariasis, leishmaniasis, and other arthropod-transmitted diseases. This work includes biochemical genetics and evaluation of cell responses and cellular defense mechanisms in both filaria-susceptible and filaria-resistant mosquitoes. In addition to mosquito and blackfly susceptibility, culicoid susceptibility is being examined with use of isoenzyme analysis and other techniques.

Studies of *Dirofilaria immitis* and its development in species of *Aedes* and *Anopheles* have focussed on the physiological, ultrastructural, and biochemical analyses of factors in the insect host cells which determine susceptibility to infection and the effects of infection on the Malphigian tubules of the mosquito. The transport physiology of the infected tubules is markedly affected in *Aedes* but not in *Anopheles* which has significance with respect to transmission potentials. There may be broader applications since a detailed ultrastructural study of mosquito osmoregulatory organs is being conducted. Relevant findings may indicate the need to evaluate the ecology of various mosquito species. Whether microfilariae can develop within a mosquito may be determined by the ability of microfilariae to avoid and/or inhibit the vector's immune response. Possibly, microfilariae avoids the vector reaction by being coated with host antigens during midgut penetration in appropriate vectors. The peritrophic appears not to function as a barrier to migration of microfilariae out of the mosquito stomach. In a related study, it was shown that the peritrophic membrane and midgut epithelium of culicoids are not barriers to *Dipetalonema* and *Mansonella* microfilarial migration out of the midgut.

Brugia pahangi infection was established in rats and mice. Subcutaneous and intraperitoneal infection routes were successful in nude mice. The thymus dependence of *B. pahangi* was established. Infections in inbred and neonate mice show that immune rejection of adult and microfilarial stages depends on presence of T cell populations. Despite the success with

Malaria Life Cycle



mouse *B. pahangi* infection it was not possible to infect nude mice with *Wuchereria bancrofti*. This indicates developmental differences in the lymphatic filariae. *Mansonella ozzardi*, a little known or understood human filaria, was established for the first time in the Patas monkey, a non-human primate. This allowed clarification of the morphology, life cycle, and taxonomic relationships of this parasite. Consequently, *Dipetalonema perstans* and *D. streptocerca*, other human filariae, are now recognized to belong to the genus *Mansonella*. The silver-tipped leaf monkey from Indonesia supports complete development of *W. bancrofti* which complements the earlier work in parasites. With the exception of *Onchocerca volvulus*, there are now animal models for all of the human filariae.

Pathogenesis and immunity

Occult infections of *B. malayi* were established in the ferret (*Mustela putorius furo*). The resultant histopathologic responses were similar to those humans with the tropical pulmonary eosinophilia syndrome. Classical Meyers-Kouwenaar (M-K) bodies develop in this host as a response to dying microfilariae. In other pathological studies on *B. malayi*, lymph thrombus formation is being analyzed. There is increased production of collagen in infected lymphatic vessel walls. The interaction of clotting factors with subendothelial collagen may influence thrombogenesis.

It was shown that neonate mice, less than 1 day old, can be infected with *B. pahangi*; they develop patent infections. Male mice developed microfilaremia; female neonates did not. Adult gravid female worms could be recovered from both sexes of mice. Neonate mice may prove very useful for evaluating immunologic parameters of infection with the lymphatic filariae. Immune responses of mammalian hosts to filarial infection were studied by characterization of the surface carbohydrates on the sheath surface of *B. pahangi* microfilariae by use of lectin binding. A comparison was made by utilizing *in vivo*-derived and immature, *in utero*-derived microfilariae. There are differences. Maturation and release of microfilariae from the uterus of adult female worms can apparently be correlated with glycosidic alterations on the sheath surface.

Genetics, in vitro cultivation, biochemistry, and chemotherapy

In a study by Japanese and U.S. scientists, the karyotypes of *B. pahangi* and *B. malayi* were analyzed and compared. Both species showed a diploid number of 10 chromosomes and by C-banding analysis the sex-determining mechanism of these species was of the XY-XX type. Both species were karyotypically indistinguishable; this further substantiates their close taxonomic relationship. Building upon prior success of *in vitro* cell line culture of third-stage larvae to the fourth stage, it is now possible to achieve propagation in a cell-free medium. This advance with lymphatic filariae will open new avenues for biochemical and immunoparasitological study. Work continued on the metabolism of adult filariae and the pathways that might be followed for development of chemotherapeutic agents against adult and larval filariae. The efficacy of diethylcarbamazine citrate (DEC) to kill *B. malayi* at various developmental stages is being evaluated. There are considerable variations in serum levels of DEC in experimental animals under constant exposure to the drug. This variation may occur in humans and account for apparent "drug failures" in some treatment programs. Studies continue on the transepidermal application of DEC. Serum levels sufficient to kill young stages of *B. malayi* develop. Work also continued on development of purified antigens potentially useful for serodiagnosis of lymphatic filariae and *Onchocerca volvulus*.

Epidemiology

Field studies in the Dominican Republic, Haiti, Ecuador, and Colombia are providing valuable information on prevalence and geographic limits of *W. bancrofti*, *Mansonella ozzardi*, and *Onchocerca volvulus*. In the Dominican Republic the highest prevalances of *W. bancrofti* positives (11.5%) occurred in the coastal plain. More than 75% of the microfilarial positives occurred in individuals under age 20. The distribution of *Onchocerca* infections, though in spotty foci, were greater than previously believed in South America.

FUTURE GOALS

Despite the considerable advances made in better understanding of diseases and their parasites, the ultimate goal of future control for either filariasis or schistosomiasis remains elusive. Evidence suggests that whereas control of one or both diseases might succeed in some limited areas the illnesses may spread in many other areas. Propagation of snail and arthropod vectors for these diseases occurs rapidly with programs to introduce water development projects into new geographic areas. This provides unfavorable environmental conditions which impact upon populations previously not troubled by such diseases. There will be a continued need for well planned epidemiological studies to determine prevalence, incidence, and intensity of schistosomiasis and filariasis in new and old foci. Acquisition of such data will allow the design of rational programs.

Panel-sponsored activities during most of the current 5-year period were limited to the original programmatic subject areas of schistosomiasis and filariasis. Future activities will be expanded. Revision of panel guidelines in 1983 and 1984 to include malaria and other vector-borne parasitic diseases will provide greater opportunities for more rapid application of new technological developments. These parasitic diseases often share common epidemiologic, ecologic, and pathogenic traits.

The striking advances over the past several years in malaria research herald the realistic possibility of a vaccine useful for prevention. Use of molecular biological techniques found helpful in malaria research should find application for filariasis, schistosomiasis, and other vector-borne diseases. Panel-sponsored research, contract activities, and free interchange at annual joint meetings has promoted rapid exchange of information, thereby enhancing the chance for better guidelines for control of such diseases. There is need for the continued encouragement and support provided by a ready source of schistosome, filarial parasites, and vectors for panel investigators. Also to be encouraged is the sponsorship of targeted projects for development of improved research methodologies, such as the cryopreservation of filarial parasites and development of molluscan cell lines. There were significant advances in development of effective drugs for schistosomiasis. Now there is need for such drugs to treat filarial infections. Promising new drugs such as the invermectins need evaluation. The prospects for effective treatment for filarial infections are excellent. Many strategies applicable for mosquito vector control will find equal use for general control of malaria and filariasis. The same approaches may apply to vectors of leishmaniasis and some of the filarial diseases.

The role of the Parasitic Diseases Panels to successfully accomplish scientific advances in these globally important, vector-borne parasitic diseases should be significant and stimulating. Collaborative efforts of the Panels and their respective scientists from Japan and the United States should provide considerable favorable progress in those many health problems caused by these parasitic diseases. The next 5-year report might well record the effective control of one or more of these devastating diseases. This is a realistic possibility.

Tropicorbis fieldi, an intermediate host found naturally infected with *S. Mansoni* in Brazil.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
GRANTS	
Nina M. Agabian University of California at Berkeley	Molecular Cloning of Schistosome Antigens
Allan R. Barr University of California at LA	Biological Control of Filariasis Vectors
Paul F. Basch Stanford University	Reproductive Biology of Schistosomes
James L. Bennett Michigan State University	Drugs or Immune Sera: Action on Schistosomules
Dov L. Boros Wayne State University	Immunoregulation of the Schistosome Egg Granuloma
Timothy J. Bradley University of California at Irvine	Cellular Defense Mechanisms in Mosquitoes

Investigator and Institution	*Title*
John P. Caulfield Brigham and Women's Hospital	*S. Mansoni*: Immune Induced Surface Changes
Bruce M. Christensen University of Wisconsin, Madison	Immune Response of Mosquitoes to Filarial Worms
Daniel G. Colley Vanderbilt University	Immunologic Responses to *Schistosoma Mansoni*
Eain M. Cornford University of California at LA	Transport Mechanisms in the Schistosome Integument
Catherine A. Crandall University of Florida	Brugian Filariasis
Raymond T. Damian University of Georgia	Worm Fecundity Suppression in Baboon Schistosomiasis
George M. Davis Academy of Natural Sciences	Analysis of Molluscan Faunas and Asian Schistosomiasis
Marc H. Dresden Baylor College of Medicine	*Schistosoma Mansoni*—Cysteinyl Proteinase of Eggs
Marc H. Dresden Baylor College of Medicine	*Schistosoma Mansoni* Acidic Proteinase of Adult Worms
Laverne K. Eveland Downstate Medical Center	Chemoattraction of *Schistosoma Mansoni in vitro*
Sharon K. File University of Puerto Rico at Piedras	Transit of Schistosome Eggs through Venular Endothelium
Ernst A. Friedheim Rockefeller University	Synthesis and Evaluation of Macrofilaricidal Compounds
Jay R. Georgi Cornell University	Migrations and Mortality of *Schistosoma*
Eugene G. Hayunga Uniformed Services University of Health Science	Characterization of Schistosome Surface Antigens
George V. Hillyer University of Puerto Rico at Piedras	Defined Immunity Antigens in Schistosomiasis
Julian J. Jaffe University of Vermont and St. Agricultural College	Folate- and Dolichol-Related Metabolism in Filariae
Stephanie L. James George Washington University	Macrophage Function in Immunity to *S. Mansoni* Infection
Thomas R. Klei Louisiana State University A & M College, Baton Rouge	Lymphatic Lesion Pathogenesis in Brugia-Infected Jirds
Paul M. Knopf Brown University	Candidate Vaccine Protein Antigens of *S. Mansoni*

Investigator and Institution	*Title*
Robert M. Lewert University of Chicago	Philippine Schistosomiasis Japonica: Immune Responses
Fred A. Lewis American Foundation for Biological Research	Immunity in a *Schistosoma Mansoni* Abortive Infection
Philip T. Loverde State University of New York at Buffalo	Cercaria-Schistosomule Transformation
Michael S. Phillips University of Pennsylvania	*In Vitro S. Mansoni* Fecundity and Granuloma Formation
Miguel J. Stadecker Tufts University	Immunoregulation in Schistosomiasis
Abram B. Stavitsky Case Western Reserve University	Immune Regulation in Schistosomiasis Japonica
Mette Strande Johns Hopkins University	Gender-Specific and Pair-Dependent Schistosome Antigens
Albert L. Vincent University of South Florida	Filariasis in the Dominican Republic
Franz C. von Lichtenberg Harvard University	Schistosomiasis and Granuloma Formation
David J. Wyler Tufts University	Fibroblast Stimulation in Schistosomiasis
Timothy P. Yoshino University of Oklahoma at Norman	Immunobiology of Schistosome-Mollusc Interactions

CONTRACTS

J. Bruce University of Lowell	Maintenance and Supply of Schistosome Infected Snails and Mammals
J. Georgi Cornell University	Radionuclide Labelling of Schistosome Cercariae and Filariid Infection Larvae
J. McCall University of Georgia	Filariasis Repository – Research Service

BIBLIOGRAPHY

Abe, T., J.T. Forbes, and D.G. Colley. Natural killer cell activity during murine schistosomiasis mansoni. *J. Parasit. 69*:1001, 1984.

Abe, T. and D.G. Colley. Modulation of *Schistosoma mansoni* egg-induced granuloma formation. III. Evidence for an anti-idiotypic, I-J positive, I-J restricted, soluble T suppressor factor. *J. Immunol. 132*:2084, 1984.

Ali, H.M., J.L. Bennett, S.M. Sulaiman, and J. Gaillot. Simple and rapid method for the determination of oltipraz, a new antischistosomal agent, in blood and urine. *J. Chromatog. 305*:465–469, 1984.

Amsden, A.F., D.L. Boros, and A.T. Hood. The etiology of the liver granulomatous response in *Schistosoma mansoni* infected athymic nude mice. *Infect. Immun. 27*:75, 1980.

Aoki, Y., A.L. Vincent, L.R. Ash, and D. Katamine. Scanning electron microscopy of third- and fourth-stage larvae and adults of *Brugia pahangi* (Nematoda: Filarioidea). *J. Parasitol. 66*:449–457, 1980.

Aronstein, W.S., and M. Strand. Lung-stage expression of a major schistosome surface antigen. *J. Parasitol. 69*:1027–1032, 1983.

Aronstein, W.S., and M. Strand. Identification of species-specific and gender-specific proteins and glycoproteins of three human schistosomes. *J. Parasitol. 69*:1006–1017, 1983.

Aronstein, W.S., and M. Strand. Gender-specific and pair-dependent glycoprotein antigens of *Schistosoma mansoni. J. Parasitol. 70*, 1983.

Ash, L.R. Recent developments in the use of animal models in studies on filariasis. *Proc. IV Cong. Latinoamericano de Parasitologia*, San Jose, Costa Rica, December, 1976, 101–108, 1980.

Attalah, A.M., F.A. Lewis, A. Urrutia-Shaw, T. Folks, and T.J. Yeatman. Natural killer cells and antibody-dependent cell-mediated cytotoxicity components of *S. mansoni* infection. *Int. Arch. Allergy. Appl. Immunol. 63*:351–355, 1980.

Barr, A.R. Cytoplasmic incompatibility in natural populations of a mosquito, *Culex pipiens* L. *Nature, 283*:71–2, 1980.

Barr, A.R. Symbiont control of reproduction in *Culex pipiens. In: Advances in Invertebrate Reproduction*, W.H. Clark and T.S. Adams, eds. Elsevier/North Holland Press, p. 382, 1981.

Barr, A.R. The *Culex pipiens* complex. In: *Cytogenetics and Genetics of Vectors*, R. Pal, J.B. Kitzmiller and T. Kanda, eds. Kodansha Ltd., Tokyo, pp. 123–136, 1981.

Barr, A.R. Symbiont control of reproduction in *Culex pipiens. In: Recent Developments in the Genetics of Insect Disease Vectors*, Stipes Publ. Co., Champaign, Ill., pp. 153–158, 1982.

Barr, A.R. The *Culex pipiens* complex. In *Recent Developments in the Genetics of Insect Disease Vectors*, Stipes Publ. Co., Champaign, Ill. (Reprinting of Barr, 1981).

Barr, A.R. Incompatibility. *In: Biocontrol of Mosquitoes*. Chap. VII(C) *Amer. Mosq. Cont. Assoc. Bull.* No. 6. In press.

Basch, P.F. Developments and behavior of *Schistosoma mansoni* fed on human erythrocyte ghosts. *Amer. J. Trop. Med. Hyg. 33*:911–917, 1984.

Basch, P.F., and M.N. Basch. Non-gametic reproductive stimulation and parthenogenesis in *Schistosoma mansoni. Parasitology 89*:369–376, 1984.

Basch, P.F., and W.R. Rhine. *Schistosoma mansoni*: Reproductive potential of male and female worms cultured *in vitro. J. Parasitol. 69*:567–569.

Bella, H., A.G.A. Rahim, M.D. Mustafa, M.A.M. Ahmed, S. Wasfi, and J.L. Bennett. Oltipraz-antischistosome efficacy in Sudanese infected with *Schistosoma mansoni. Amer. J. Trop. Med. 31*:775–778, 1982.

Bennett, J.L. Characteristics of an antischistosomal benzodiazepine receptor in *Schistosoma mansoni. J. Parasitol. 66*:742–747, 1980.

Bennett, J.L., and J.W. Depenbusch. Recent advances in the chemotherapy of schistosomiasis. In *Parasitic Diseases, Vol. 2. The Chemotherapy*. J.M. Mansfield (ed.), Marcel Dekker, Inc., New York, 1984, pp. 73–132.

Bentley, A.G., A.S. Carlisle, and S.M. Phillips. Ultrastructural analysis of the cellular response to *Schistosoma mansoni*. Initial and challenge infections in the rat. *Amer. J. Trop. Med. Hyg. 30*:102, 1981.

Bentley, A.G., A.S. Carlisle, and S.M. Phillips. Ultrastructural analysis of the cellular response to *Schistosoma mansoni*. II. Inflammatory responses in rodent skin. *Amer. J. Trop. Med. Hyg. 30*:815, 1981.

Bentley, A.G., B.L. Doughty, and S.M. Phillips. Ultrastructural analysis of the cellular response to Schistosomiasis mansoni. III. The *in vitro* granuloma. *Amer. J. Trop. Med. Hyg. 31(6)*:1168, 1982.

Bocash, W.D., E.M. Cornford, and W.H. Oldendorf. *Schistosoma mansoni*: Correlation between lipid partition coefficients and transintegumental uptakes of nonelectrolytes. *Exp. Parasitol. 52*:396–403, 1981.

Bogitsh, B.J., and M.H. Dresden. Fluorescent microscopy of acid proteases in adult *Schistosoma mansoni* and *Schistosoma japonicum. J. Parasitol. 69*:106, 1983.

Boros, D.L., A.F. Amsden, and A.T. Hood. Modulation of granulomatous hypersensitivity. IV. Immunoglobulin and antibody production by vigorous and immunomodulated liver granulomas of *Schistosoma mansoni*-infected mice. *J. Immunol.* *128*:1050, 1982.

Boros, D.L., and M.A. Lande. Induction of collagen synthesis in cultured human fibroblasts by live *S. mansoni* eggs and soluble egg antigens (SEA). *Amer. J. Trop. Med. Hyg.* *32*:73, 1983.

Boros, D.L., M.A. Lande, and L. Carrick, Jr. The influence of host and parasite factors on *Schistosoma mansoni* egg-induced tissue fibrosis. *Contributions Microbiol. Immunol.* *7*:230, 1983.

Bradley, T.J. Mitochondrial placement and function in insect ion-transporting cells. *Amer. Zool.* *24*:157-167, 1984.

Bradley, T.J. The Excretory System: Structure and Physiology. In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. G.A. Kerkut and L.I. Gilbert, eds. Pergamon Press, Oxford, New York. In press.

Bradley, T.J., and T.A. Miller, eds. *Measurement of Ion Transport and Metabolic Rate in Insects*. Springer-Verlag, N.Y. 288 pp. 1983.

Bradley, T.J. and J.K. Nayar. The effect of infection with *Dirofilaria immitis* (Dog Heartworm) on fluid secretion rates in the Malpighian tubules of the mosquitoes *Aedes taeniorhynchus* and *Anopheles quadrimaculatus*. *J. Insect Physiol.* In press.

Bradley, T.J. and J.K. Nayar. Intracellular melanization of the larvae of *Dirofilaria immitis* in the Malpighian tubules of the mosquito, *Aedes sollicitans*. *J. Invert. Path.* In press.

Bradley, T.J., D.M. Sauerman, Jr., and J.K. Nayar. Early cellular responses in the Malpighian tubules of the mosquito *Aedes taeniorhynchus* to infection with *Dirofilaria immitis* (Dog Heartworm). *J. Parasitol.* *70*:82-88, 1984.

Bradley, T.J., K. Strange, and J.E. Phillips. Osmotic and ionic regulation in saline-water mosquito larvae. In: *Osmoregulation in Estuarine and Marine Animals*. A Pequeux, R. Gilles & L. Bolis, eds. Springer Verlag, Berlin, Heidelberg, New York. 1984.

Bradley, T.J., A.M. Stuart, and P. Satir. The ultrastructure of the larval Malpighian tubules of the saline-water mosquito, *Aedes taeniorhynchus*. *Tissue and Cell.* *14*:759-773, 1982.

Byram, James E., and F. von Lichtenberg. Experimental infection with *Schistosoma mekongi* in laboratory animals: Parasitological and pathological findings. *In: Mekong Schistosomiasis*. J.I. Bruce and C. Harinasuta, eds. University of Michigan Press, Ann Arbor, 1980.

Byram, J.E., F. von Lichtenberg, F.A. Lewis, and M.A. Stirewalt. Pathology of a live attenuated anti-schistosome vaccine. *Amer. J. Trop. Med. Hyg.* *32*:96-108, 1982.

Carter, C.E. and D.G. Colley. *Schistosoma japonicum* soluble egg antigen: Separation by con A chromatography immunoaffinity purification. *Mol. Immunol.* *18*:219, 1981.

Caulfield, J.P., A. Hein, G. Moser, and A. Sher. Light and electron microscopic appearance of rat peritoneal mast cells adhering to schistosomula of *Schistosoma mansoni* by means of complement or antibody. *J. Parasitol.* *67*:776-783, 1981.

Caulfield, J.P., G. Korman, A.E. Butterworth, M. Hogan, and J.R. David. The adherence of human neutrophils and eosinophils to schistosomula: Evidence for membrane fusion between cells and parasites. *J. Cell Biol.* *86*:46-63, 1980.

Caulfield, J.P., G. Korman, A.E. Butterworth, M. Hogan, and J.R. David. Partial and complete detachment of neutrophils and eosinophils from schistosomula: Evidence for the establishment of continuity between a fused and normal parasite membrane. *J. Cell Biol.* *86*:64-76, 1980.

Caulfield, J.P., G. Korman, and J.C. Samuelson. Human neutrophils endocytose multivalent ligands from the surface of schistosomula of *S. mansoni* before membrane fusion. *J. Cell Biol.* *94*:370-378, 1982.

Chensue, S.W., D.L. Boros, and C.S. David. Regulation of granulomatous inflammation in murine schistosomiasis: *In vitro* characterization of T lymphocyte subsets involved in the production and suppression of migration inhibitory factor (MIF). *J. Exp. Med.* *151*:1398, 1980.

Chensue, S.W., D. L. Boros, and C.S. David. Regulation of granulomatous inflammation in murine schistosomiasis. II. T suppressor cell-derived, I-C subregion-encoded soluble suppressor factor mediates regulation of lymphokine production. *J. Exp. Med.* *157*:219, 1983.

Chensue, S.W., S.L. Kunkel, G.I. Higashi, P.A. Ward, and D.L. Boros. Production of superoxide anion, prostaglandins and hydroxyeicosa-tetraenoic acids by macrophages from hypersensitivity *Schistosoma mansoni* egg and foreign body-type granulomas. *Infect. Immun.* *42*:1116, 1983.

Chensue, S.W., S.R. Wellhausen, and D.L. Boros. Modulation of granulomatous hypersensitivity. II. Participation of Ly1^{+} and Ly2^{+} T lymphocytes in the suppression of granuloma formation and lymphokine production in *Schistosoma mansoni*-infected mice. *J. Immunol.* *127*:363, 1981.

Christensen, B.M. A taxonomic review of the genus *Loxogenoides (Digenea:Lecithodendriidae)* with a description of *Loxogenoides loborchis* sp. n. from *Rana catesbeiana* Shaw in western Kentucky. *Proc. Helminthol. Soc. Wash.* *48*:65-70, 1981.

Christensen, B.M. Effect of *Dirofilaria immitis* on the fecundity of *Aedes trivittatus*. *Mosquito News* *41*:78-81, 1981.

Christensen, B.M. Observations on the immune response of *Aedes trivittatus* against *Dirofilaria immitis*. *Trans. Roy. Soc. Trop. Med. Hyg.* *75*:439-443.

Christensen, B.M. and D.R. Sutherland. *Brugia pahangi*: Exsheathment and midgut penetration in *Aedes aegypti*. *Trans. Amer. Microscop. Soc. 103*:423–433, 1984.

Christensen, B.M., D.R. Sutherland, and L.N. Gleason. Defense reactions of mosquitoes to filarial worms: Comparative studies on the response of three mosquitoes to inoculated *Brugia pahangi* and *Dirofilaria immitis* microfilariae. *J. Invert. Pathol.* In press.

Christensen, B.M., P.K. Wellner, and L. Gleason. Two new species of *Lissorchis* (Digenea: Lissorchiidae) and *Minytrema melanops*, with a key to species. *Proc. Helminthol. Soc. Wash. 49*:22–27, 1982.

Chuah, S.-K. and A.R. Barr. Preparation of antibody to *Wolbachia pipientis* of *Culex pipiens*. *Mosquito News, 41*:639-642, 1981.

Colley, D.G. Lymphokine-related eosinophil responses. In: *Lymphokine Reports. 1*:135–155. Academic Press, 1980.

Colley, D.G. Lymphocyte Products. *In: The Eosinophil in Health and Disease*, eds. A.A.F Mahmoud and K.F. Austen, Grune and Stratton, pp. 293–309, 1980.

Colley, D.G., T. lymphocytes which contribute to the immunoregulation of granuloma formation in chronic murine schistosomiasis. *J. Immunol. 126*:1465, 1981.

Comley, J.C.W. and J.J. Jaffe. Isoprenoid biosynthesis in adult *Brugia pahangi* and *Dirofilaria immitis*. *J. Parasitol. 67*:609-616, 1981.

Comley, J.C.W., J.J. Jaffe, L.R. Chrin, and R.B. Smith. Synthesis of ubiquinone 9 by adult *Brugia pahangi* and *Dirofilaria immitis*. Evidence against its involvement in the oxidation of 5-methyltetrahydrofolate. *Mol. Biochem. Parasitol. 2*:271–283, 1981.

Compton, C.C. and F. von Lichtenberg. Necrotizing granulomatous gastritis and gastric perforation of unknown etiology. *J. Clin. Gastroenterol.* 5:59–65, 1983.

Cornford, E.M. Correlation between lipid partition coefficients and the surface permeation in *Schistosoma japonicum*. *J. Memb. Biol. 64*:217–224, 1982.

Cornford, E.M. Differential uptake of 3-0 methyl glucose in paired and unpaired schistosomes. *In: "Nuclear Techniques in the Study of Parasitic Infections,"* International Atomic Energy Agency, Vienna, pp. 497–502, 1982.

Cornford, E.M., W.D. Bocash, and W.H. Oldendorf. Transintegumental glucose uptake in *Schistosomatium douthitti*. *J. Parasitol.* 67:24–30, 1981.

Cornford, E.M., L.D. Braun, W.D. Pardridge, and W.H. Oldendorf. Blood flow rate and cellular influx of glucose and arginine in mouse liver *in vivo*. *Amer. J. Physiol. 238*:H553–H560, 1980.

Cornford, E.M., P.D. Crane, L.D. Braun, W.D. Bocash, A.M. Nyerges, and W.H. Oldendorf. Reduction in brain glucose utilization rate after tryptophol (3-indole ethanol) treatment. *J. Neurochem. 36*:1758–1765, 1981.

Cornford, E.M., C.P. Diep, and G.A. Rowley. *Schistosoma mansoni, S. japonicum, S. haematobium*: Glycogen content and glucose uptake in parasites from fasted and control hosts. *Exp. Parasitol.* 56:397–408, 1983.

Cornford, E.M. and M.E. Huot. Glucose transfer from male to female schistosomes. *Science 213*:1269–1271, 1981.

Cornford, E.M., M.E. Huot, C.P. Diep, and G.A. Rowley. Protein, glycogen and water content in schistosomes. *J. Parasitol.* 68:1010–1020, 1982.

Courtney, C.C. and B.M. Christensen. Diversity and seasonal abundance of mosquitoes (Diptera:Culicidae) in Calloway County, Kentucky. *Trans. Ky. Acad. Sci. 43*:55–59, 1982.

Courtney, C.C. and B.M. Christensen. Field isolations of filarial worms presumed to be *Dirofilaria immitis* from *Anopheles punctipennis* in Kentucky. *Mosquito News 43*:366–368, 1983.

Courtney, C.C., B.M. Christensen, and W.G. Goodman. Effect of *Dirofilaria immitis* on blood meal size and fecundity in *Aedes aegypti*. *J. Med. Entomol.* In press.

Crandall, R.B., C.A. Crandall, J.T. Neilson, J.T. Fletcher, W.W. Kozek, and B. Redington. Antibody responses to experimental *Brugia malayi* infections in Patas and Rhesus monkeys. *Acta Trop. 40*:53–64, 1983.

Crandall, R.B., P.B. McGrevy, D.H. Connor, C.A. Crandall, J.T.M. Neilson, and J.W. McCall. The ferret *(Mustela putorious furo)* as an experimental host for *Brugia malayi* and *Brugia pahangi*. *Amer. J. Trop. Med. Hyg. 31*:752–759, 1982.

Crandall, C.A., J.T.M. Neilson, and R.B. Crandall. Evaluation of inbred strains of hamsters as hosts of *Brugia malayi*. *Trans. Roy. Soc. Trop. Med. Hyg.* 76:277, 1982.

Crandall, R.B., J.P. Thompson, D.H. Connor, P.B. McGreevy, and C.A. Crandall. Pathology of experimental infection with *Brugia malayi* in ferrets: Comparison with occult filariasis in man. *Acta Trop.* In press.

Cross, J.H., F. Partono, M.Y.-K Hsu, L.R. Ash, and S. Oemijati. Further studies on the development of *Wuchereria bancrofti* in laboratory animals. *Southeast Asian J. Trop. Med. Pub. Hlth. 12*:114–122, 1981.

Dalesandro, D.A., and R.T. Damian. Mitogenic responses of baboon *(Papio cynocephalus)* peripheral blood mononuclear cells. A possible role for monocytes. *Vet. Immunol. Immunopathol.* In press.

Damian, R.T. and R.W. Chapman. The fecundity of *Schistosoma mansoni* in baboons, with evidence for a sex ratio effect. *J. Parasitol.* 69:987–989, 1983.

Damian, R.T., N.D. Green, T. Suzuki, and D.A. Dean. *Schistosomiasis mansoni* in baboons. V. Antibodies and delayed hypersensitivity in multiply infected *Papio cynocephalus*. *Amer. J. Trop. Med. Hyg. 30*:836–843, 1981.

Damian, R.T., M.R. Powell, M.L. Roberts, J.D. Clark, M.A. Stirewalt, and F.A. Lewis. *Schistosoma mansoni*: Parasitology and immunology of baboons vaccinated with irradiated cryopreserved schistosomula. *Int. J. Parasitol.* In press.

Damian, R.T., M.L. Roberts, M.R. Powell, J.D. Clark, F.A. Lewis, and M.A. Stirewalt. *Schistosoma mansoni* egg granuloma size reduction in challenged baboons after vaccination with irradiated cryopreserved schistosomula. *Proc. Nat. Acad. Sci. 81*:3552–3556, 1984.

Damian, R.T., M.R. Powell, M.L. Roberts, J.D. Clark, M.A. Stirewalt, and F.A. Lewis. *Schistosoma mansoni*: Parasitology and immunology of baboons vaccinated with irradiated cryopreserved schistosomula. *Int. J. Parasitol.* In press.

Davis, G.M. Snail hosts of Asian *Schistosoma* infecting man: Evolution and co-evolution. In: Bruce, ed., *The Mekong Schistosome*, *Malacol. Rev.* Supplement No. 2, pp. 195–238.

Davis, G.M. Introduction to the Second International Symposium on Evolution and Adaptive Radiation of Mollusca. *Malacologia 21*:1–4, 1981.

Davis, G.M. Different modes of evolution and adaptive radiation in the Pomatiopsidae (Prosobranchia:Mesogastropoda). Proceedings of the Second International Symposium on Molluscan Evolution and Adaptive Radiation, Perpignan, France. *Malacologia 21*:209–262, 1981.

Davis, G.M. Historical and ecological factors in the evolution, adaptive radiation, and biogeography of freshwater molluscs. *Amer. Zool. 22*:375–395, 1982.

Davis, G.M. *Spurwinkia*: Morphology, systematics, and ecology of a new genus of North American Marshland Hydrobiidae (Mollusca:Gastropoda). *Proc. Acad. Nat. Sci. Phila. 134*:143–177, 1982.

Davis, G.M. *Poamolithus*: Morphology, convergence and relationships among Hydrobioid snails. *Malacologia, 25*:73–108, 1983.

Davis, G.M. and G. Greer. A new genus and two new species of triculinae (Gastropoda:Prosobranchia) and the transmission of a Malayasian mammalian *Schistosoma* sp. *Pro. Acad. Nat. Sci. Phila. 132*:245–276, 1980.

Davis, G.M. and R. Hershler. The morphology of *Hydrobia truncata* (Gastropoda:Hydrobiidae): Relevance to systematics of *Hydrobia*. *Biol. Bull. 158*:195–219, 1980.

Davis, G.M. and P.W. Kat. Speciation in molluscs from Turkane Basin. *Nature, 304*:660–661.

Davis, G.M., Y. Kuo, K.E. Hoagland, P. Chen, H. Yang, and D. Chen. Advances in systematics of the Triculinae: The genus *Fenouilia* of Yunnan, China. *Proc. Acad. Nat. Sci. Phila. 135*:177–199, 1983.

Davis, G.M., Y. Kuo, K.E. Hoagland, P. Chen, H. Yang, and D. Chen. *Kunmingia*: A new genus of Triculinae (Gastropoda:Pomatiopsidae) from China: phenetic and cladistic relationships. *Proc. Acad. Nat. Sci. Phila.* In press.

Dean, D.A., B.L. Mangold, J.R. Georgi, R.H. Jacobson. Comparison of *Schistosoma mansoni* migration patterns in normal and irradiated cercaria-immunized mice by means of autoradiographic analysis. *Amer. J. Trop. Med. Hyg. 33*:89–96, 1984.

Depenbusch, J.W., D.P. Thompson, R.A. Pax, and J.L. Bennett. Tegumental disruption with Triton X-100 and its effects on longitudinal muscle function in male *S. mansoni*. *Parasitology 87*:61–73, 1983.

Devine, D.V. and W.M. Kemp. Immunocytochemical localization molluscan host-like antigenic determinants on the surfaces of adult *Schistosoma mansoni*. *J. Parasitol. 70*:172–174, 1984.

Dotson, M.J., S.H. Chu, and G.R. Hillman. Selective inhibition of schistosome acetylcholinesterase by dansylated acetylcholine analogs. *Comp. Biochem. Physiol. 68C*:229–230, 1981.

Doughty, B.L., E.A. Ottesen, T.E. Nash, and S.M. Phillips. Delayed hypersensitivity granuloma formation around *Schistosoma mansoni* eggs *in vitro*. *J. Immunol. 133*:993–997, 1984.

Doughty, B.L. and S.M. Phillips. Delayed hypersensitivity granuloma formation around *Schistosoma mansoni* eggs *in vitro*. I. Definition of the model. *J. Immunol. 128*:30, 1982.

Doughty, B.L. and S.M. Phillips. Delayed hypersensitivity granuloma formation and modulation around *Schistosoma mansoni* eggs *in vitro*. II. Regulatory T-cell subsets. *J. Immunol. 128*:37, 1982.

Doughty, B.L., D.M. Zodda, A.E. Kholy, and S.M. Phillips. Delayed type hypersensitivity granuloma formation around *Schistosoma mansoni* eggs *in vitro*. IV. Granuloma formation in human schistosomiasis. *Amer. J. Trop. Med. Hyg.* In press.

Dresden, M.H. Proteolytic enzymes of *Schistosoma mansoni*. *Acta Leidensia 49*:81, 1982.

Dresden, M.H. and D.C. Payne. A sieving method for the collection of schistosome eggs from mouse intestines. *J. Parasitol. 67*:450, 1981.

Dresden, M.H., D.C. Payne, and P.F. Basch. Acidic thiol proteinase activity of *Schistosoma mansoni* cultured *in vitro*. *Mol. Biochem. Parasitol. 6*:203–208, 1982.

Dresden, M.H., J.P. Rotmans, A.M. Deelder, G. Koper, and J.S. Ploem. Automated measurement of proteinase activity with a fluorogenic substrate using an inverted fluorescence microscope. *Anal. Biochem. 126*:170, 1982.

Dresden, M.H., M.L. Rutledge, and C.L. Chappell. Properties of the acidic thiol proteinase from *Schistosoma mansoni* adults. *Biochem. Mol. Parasitol.* 4:61, 1981.

Dresden, M.H., C.K. Sung, and A.M. Deelder. A monoclonal antibody from infected mice to the thiol-dependent proteinase of *Schistosoma mansoni* eggs. *J. Immunol. 130*:1, 1983.

Duhrkopf, R.E. Low-level autogeny in a strain of *Aedes polynesiensis* Marks from Fiji. *Mosquito News 40*:633–634, 1980.

Duhrkopf, R.E. and M. Trpis. The degree of susceptibility and levels of infection in ten different strains of *Aedes polynesiensis* Marks infected with subperiodic *Brugia malayi* and *Brugia pahangi*. *Amer. J. Trop. Med. Hyg. 29*:815–819, 1980.

Duhrkopf, R.E. and M. Trpis. The effect of tetracycline treatment on filarial susceptibility in members of the *Aedes scutellaris* complex. *Mosquito News 41*:729–732, 1981.

Ehrlich, H.P. and D.J. Wyler. Fibroblast contraction of collagen lattices *in vitro:* Inhibition by chronic inflammatory cell mediators. *J. Cell. Physiol. 116*:345–351, 1983.

El-Naggar, A. and D.G. Colley. Modulation of *Schistosoma mansoni* egg-induced granuloma formation. II. Soluble suppressor activity from lymphoid cells during chronic infection. *Cell. Immunol. 72*:151, 1982.

Elliott, D.E. and D.L. Boros. Schistosome egg antigen(s) presentation and regulatory activity by macrophages isolated from vigorous or immunomodulated liver granulomas of *Schistosoma mansoni*-infected mice. *J. Immunol. 132*:1506, 1984.

Eveland, L.K., B. Fried and L.M. Cohen. *Schistosoma mansoni*: Adult worm chemoattraction with and without barriers. *Exp. Parasitol. 54*:271–276, 1982.

Eveland, L.K., B. Fried, and L.M. Cohen. *Schistosoma mansoni*: Adult worm chemoattraction with barriers of specific molecular weight exclusions. *Exp. Parasitol. 56*:255–258, 1983.

Fetterer, R.H., R.A. Pax, and J.L. Bennett. Praziquantel, potassium and 2,4-dinitrophenol: Analysis of the reaction on the musculature of *Schistosoma mansoni*. *Amer. J. Pharmacol. 64*:31–38, 1980.

Fetterer, R.H., R.A. Pax, and J.L. Bennett. Na+ -K+ transport, contractile activity and the tegumental membrane potential of *S. mansoni*. *Parasitol. 82*:97–109, 1980.

Fetterer, R.H., R.A. Pax, D. Thompson, C. Bricker, and J.L. Bennett. Praziquantel: Mode of its antischistosomal action. *In: The Host Invader Interplay* (H. Van den Bossche, ed.), Elsevier/North-Holland, Amsterdam, 1980.

Fetterer, R.H., R.A. Pax, D. Thompson, C. Bricker, and J.L. Bennett. Electrophysiological and biochemical properties of the tegument of *Schistosoma mansoni*. *In: The Host Invader Interplay* (H. Van den Bossche, ed.), Elsevier/North-Holland, Amsterdam, 1980.

Fetterer, R.H., E. Wolde Mussie, and J.L. Bennett. Adenosine triphosphate-dependent ouabain binding in homogenates of male and female *Schistosoma mansoni*. *Comp. Biochem. Physiol. 69*:803–808, 1981.

Files, S.K. and P. Davis. A method for preparing cells growing on a collagen substrate for scanning electron microscopy. *Texas Soc. Electron Microscopy. 14*:No. 2.

Fletcher, M., P.T. LoVerde, and R.E. Kuntz. Electrophoretic differences between *Schistosoma mansoni* and *S. rodhaini*. *J. Parasitol. 67*:593–595, 1981.

Fried, B., L.K. Eveland, and Lee M. Cohen. Survival and pairing of *Schistosoma mansoni* on the chick chorioallantois. *Proc. Helm. Soc. Wash. 49*:330–331, 1982.

Fried, B., P.S. Imperia, and L.K. Eveland. Neutral lipids in adult male and female *Schistosoma mansoni* and release of neutral lipids by adults maintained *in vitro*. *Comp. Biochem. Physiol. 66B*:111–112, 1980.

Fried, B., J. Shenko, and L.K. Eveland. Densitometric thin-layer chromatographic analyses of cholesterol in *Schistosoma mansoni* (Trematoda) adults and their excretory-secretory products. *J. Chem. Ecol.* 9:1483–1489, 1982.

Friedheim, E. Trypanocidal and filaricidal melanimyl thioarsenides (MTAs). *WHO/Fil/Sng* 7:82, 1982.

Friedheim, E., C. Corvi, J. Graziano, T. Donnelly, and D. Breslin. Choroid plexus as protective sink for heavy metals? *Lancet*, 981, 1983.

Furman, A. and L.R. Ash. Analysis of *Brugia pahangi* microfilariae surface carbohydrates: Comparison of the binding of a panel of fluoresceinated lectins to mature *in vivo*-derived and immature in utero-derived microfilariae. *Acta Tropica, 40*:45–51, 1983.

Furman, A. and L.R. Ash. Infections of *Brugia pahangi* in neonate mice. *J. Parasitol 69*:441–442, 1983.

Furman, A. and L.R. Ash. Parameters influencing the susceptibility of neonate mice to infection with *Brugia pahangi*. *J. Parasitol 69*:1038–1042, 1983.

Furman, A. and L.R. Ash. Characterization of the exposed carbohydrates on the sheath surface of *in vitro*-derived *Brugia pahangi* microfilariae by analysis of lectin binding. *J. Parasitol 69*:1043–1047, 1983.

Garb, K.S. and A.B. Stavitsky. Depressed *in vitro* and *in vivo* antibody response and adoptive transfer of delayed hypersensitivity to myoglobin with spleen cells of mice chronically infected with *Schistosoma japonicum* and injected with myoglobin. *Infect. Immun. 43*:1097, 1984.

Garrett, M.A. and T.J. Bradley. Osmoregulation and rectal ultra-structure in *Culiseta inornata*. *Amer. Zool. 22*:914, 1982.

Garrett, M.A. and T.J. Bradley. Saline tolerance and osmoregulation of larvae of a brackish-water mosquito, *Culex tarsalis*. *Mosquito Control Research Ann. Rep.*, University of California, pp. 32-33. 1983.

Garrett, M. and T.J. Bradley. The pattern of osmotic regulation in larvae of the mosquito *Culiseta inornata*. *J. Exp. Biol.* In press.

Garrett, M.A. and T.J. Bradley. Ultrastructure of osmoregulatory organs in larvae of the brackish-water mosquito, *Culiseta inornata* (Williston). *J. Morph.* In press.

Georgi, J.R. *Schistosoma mansoni*: Quantification of skin penetration and early migration by differential external radioassay and autoradiography. *Parasitology 84*:263-281. 1982.

Georgi, J.R., D.A. Dean, and B.L. Mangold. *Schistosoma mansoni*: Temporal distribution of radioselenium-labeled schistomula in lungs of mice during the first two weeks of infection. *Parasitology 86*:31-36. 1983.

Gittelman, H.G., R.B. Grieve, M.M. Hitchings, R.H. Jacobson and R.H. Cypess. Quantitative fluorescent immunoassay for measurement of antibody to *Dirofilaria immitis* in dogs. *J. Clin. Microbiol. 13*:309-312. 1981.

Gitter, B.D. and R.T. Damian. Murine alloantigen acquisition by schistosomula of *Schistosoma mansoni*: Further evidence for the presence of K, D, and I region gene products on the tegumental surface. *Parasite Immunology 4*:383-393. 1982.

Gitter, B.D., S.L. McCormick and R.T. Damian. Murine alloantigen acquisition by *Schistosoma mansoni*: Presence of H-2K determinants on adult worms and failure of allogeneic lymphocytes to recognize acquired MHC gene products on schistosomula. *J. Parasitol 68*:513-518. 1982.

Glickman, L.T., R.B. Grieve, E.B. Breitschwerdt, M. Mika-Grieve, G.J. Patronek, L.M. Domanski and C. Root. Serologic pattern of canine heartworm (*Dirofilaria immitis*) infection. *Amer. J. Vet. Res. 45*:1178-1183. 1984.

Granath, Jr., W.O. Intracellular distribution of lysosomal enzymes within the hemocytes of *Biomphalaria glabrata*. *Trans. Amer. Microsc. Soc. 103*:38-43. 1984.

Granath, Jr., W.O. and T.P. Yoshino. Characterization of molluscan phagocyte subpopulations based on lysosomal enzyme markers. *J. Exp. Zool. 226*:205-210. 1983.

Granath, Jr., W.O. and T.P. Yoshino. Lysosomal enzyme activities in susceptible and refractory strains of *Biomphalaria glabrata* during the course of infection with *Schistosoma mansoni*. *J. Parsitol. 69*:1018-1026. 1984.

Granath, Jr., W.O. and T.P. Yoshino. *Schistosoma mansoni*: Passive transfer of resistance by serum in the vector snail, *Biomphalaria glabrata*. *Exp. Parasitol. 58*:188-193. 1984.

Green, W.F. and D.G. Colley. Modulation of *Schistosoma mansoni* egg-induced granuloma formation: I-J restriction of T cell-mediated suppression in a chronic parasitic infection. *Proc. Natl. Acad. Sci. 78*:1152. 1981.

Grieve, R.B. Serology and serodiagnosis of canine dirofilariasis. *Proc. Heartworm Symp.* 1980. *Veterinary Medicine Publishing Company*, Edwardsville, Kansas. pp. 37-40. 1981.

Grieve, R.B. Immunodiagnosis of heartworm infection: An update. *Proc. Heartworm Symp.* 1983. *Veterinary Medicine Publishing Company*. Edwardsville, Kansas. pp. 44-47. 1984.

Grieve, R.B., B.O. Brooks, J.G. Babish, R.H. Jacobson, and R.H. Cypess. Lymphocyte function in experimental canine dirofilariasis: B-cell responses to heterologous antigen. *J. Parasitol. 68*:341-343. 1982.

Grieve, R.B., K.T. DeGregory, and D.L. Lindmark. Extraction and characterization of surfactant-soluble antigens from adult female *Dirofilaria immitis*. *Acta Tropica*. In press.

Grieve, R.B. and D.H. Knight. Anti-*Dirofilaria immitis* antibody levels before and after anthelmintic treatment of experimentally infected dogs. *J. Parasitol.* In press.

Grieve, R.B. and S. Lauria. Periodicity of *Dirofilaria immitis* microfilariae in canine and murine hosts. *Acta Tropica 40*:121-127. 1983.

Grieve, R.B., J.B. Lok, and L.T. Glickman. Epidemiology of canine heartworm infection. *Epidemiol. Reviews 5*:220-246. 1983.

Grieve, R.B., M. Mika-Grieve, J.B. Lok, T.F. Marchell, and E.W. Cupp. Recovery and viability of *Dirofilaria immitis microfilariae*. *Acta Tropica 41*:271-278. 1984.

Grieve, R.B., M. Mika-Johnson, R.H. Jacobson, and R.H. Cypess. Enzyme-linked immunosorbent assay for measurement of antibody responses to *Dirofilaria immitis* in experimentally infected dogs. *Amer. J. Vet. Research 42*:66-69. 1981

Grossman, A.I., R.B. Short, and R.E. Kuntz. Somatic chromosomes of *Schistosoma rodhaini*, *S. mattheei*, and *S. intercalatum*. *J. Parasitol. 67*:41-44. 1981.

Guptavanij, P. and A.R. Barr. Two sex-linked mutants, *blond* (bl) and *maroon eye* (mar), in *Culex pipiens* (Diptera: Culicidae). *J. Med. Entomol. 19*:394-8. 1982

Guptavanij, P. and A.R. Barr. Failure of culicine eggs to darken in the field. *J. Med. Entomol.* In press.

Haseeb, M.A., L.K. Eveland, and B. Fried. Histochemical lipid studies on *Schistosoma mansoni* adults maintained *in situ* and *in vitro*. *Int. J. Parasitol. 14*:83-88. 1983.

Hasseb, M.A., L.K. Eveland, B. Fried, and M.A. Hayat. Transmission electron microscopic observations on lipid release in *Schistosoma mansoni* maintained *in vitro*. *Int. J. Parasitol.* In press.

Haseeb, M.A., B. Fried, and L.K. Eveland. *Schistosoma haematobium*: Neutral lipid composition and release by adults maintained *in vitro*. *Comp. Biochem. Physiol.* In press.

Hayunga, E.G. Radiolabeling of surface antigens on adult schistosomes. *In*: G.T. Strickland and K.W. Hunter, eds. *Immunoparasitology: Principles and Methods in Malaria and Schistosomiasis Research*. *Praeger Scientific*, New York, pp. 279-291.

Hayunga, E.G. Isolation and purification of schistosome antigens by affinity chromatography using immobilized lectins. *Atomic Energy Applications in Parasitology*. International Atomic Energy Agency, Vienna, Austria. In press.

Hayunga, E.G. and K.D. Murrell. Some problems associated with radiolabeling surface antigens on helminth parasites: A brief review. *Vet. Parasitol.* *10*:205-219, 1982.

Hayunga, E.G., M.P. Sumner, and M. Stek, Jr. Isolation of a concanavalin A binding glycoprotein from adult *Schistosoma japonicum*. *J. Parasitol* *68*:960-961, 1982.

Hayunga, E.G., M.P. Sumner, M. Stek, Jr., W.E. Vannier, and R.Y. Chesnut. Purification of the major concanavalin A binding surface glycoprotein from adult *Schistosoma mansoni*. *Proc. Helm. Soc. Wash.* *50*:219-235, 1983.

Hayunga, E.G., M.P. Sumner, and W.E. Vannier. Radiolabeling, extraction and characterization of surface antigens as illustrated by studies on *Schistosoma mansoni*. *Atomic Energy Applications in Parasitology*. International Atomic Energy Agency, Vienna, Austria. In press.

Hayunga, E.G., W.E. Vannier, and R.Y. Chesnut. Partial characterization of radiolabeled antigens from adult *Schistosoma haematobium*. *J. Parasitol.* *67*:589-591, 1981.

Hillman, G.R., S.-H Chu, and M.J. Dotson. Effects of dansylated acetylcholine analogs on *Schistosoma mansoni*. *J. Pharm. Sci.* *69*:516-520, 1980.

Hillman, G.R. and D.A. Johnston. Histochemical labeling of drug receptors in schistosomes: Quantitation using computer image analysis techniques. *J. Pharm. Methods* 9:269-277, 1983.

Hood, A.T. and D.L. Boros. The effect of splenectomy on the pathophysiology and egg-specific immune response to *S. mansoni* infected mice. *Amer. J. Trop. Med. Hyg.* 29:586, 1980.

Imperia, P.S., B. Fried, and L.K. Eveland. Pheromonal attraction of *Schistosoma mansoni* females towards males in the absence of worm-tactile behavior. *J. Parasitol.* *66*:682-684, 1980.

Irie, Y., P.F. Basch, and M.N. Basch. Ultrastructure of the reproductive organs of *Schistosoma mansoni* cultured from cercariae. *J. Parasitol.* 6:559-566, 1983.

Jaffe, J.J. Filarial folate-related metabolism as a potential target for selective inhibitors. *In: The Host Invader Interplay* (H. Van den Bossche, ed.) *Elsevier North-Holland Biomedical Press*, Amsterdam 605-614, 1980.

Jaffe, J.J. and L.R. Chrin. Folate metabolism in filariae. Enzymes associated with 5,10-methylenetetrahydrofolate. *J. Parasitol.* *66*:53-58, 1980.

Jaffe, J.J. and L.R. Chrin. Involvement of tetrahydrofolate cofactors in de novo purine ribonucleotide synthesis by adult *Brugia pahangi* and *Dirofilaria immitis*. *Mol. Biochem. Parasitol.* 2:259-270, 1981.

Jaffe, J.J., L.R. Chrin, and R.B. Smith. Folate metabolism in filariae. Enzymes associated with the formation of 10-formyltetrahydrofolate. *J. Parasitol.* *66*:428-433, 1980.

Jaffe, J.J., L.R. Chrin, and R.B. Smith. 5,10-Methenyltetrahydrofolate cyclohydrolase and 5-formyltetrahydrofolate cyclodehydrase in *Aedes aegypti*. *Comp. Biochem. Physiol.* *66B*:597-600, 1980.

Jaffe, J.J., R.A. Lambert, and W.P. Tong. Formation, retention and enzyme inhibitory activity of methotrexate polyglutamates in adult *Brugia pahangi*. *In*: *Folyl and Antifolyl Polyglutamates*, Vol. 2 (I.D. Goldman, B.A. Chabner, and J.R. Bertino, eds.), *Praeger Scientific*, Allentown. In press.

James, S.L. Induction of protective immunity against *Schistosoma mansoni* by a nonliving vaccine is dependent upon the method of antigen presentation. *J. Immunol.* In press.

James, S.L., R. Correa-Oleivera, and E.J. Leonard. Defective vaccine-induced immunity to *Schistosoma mansoni* in P strain mice. II. Analysis of cellular responses. *J. Immunol.* *133*:1587-1593, 1984.

Jeffers, G.W., T.R. Klei, and F.M. Enright. Activation of jird (*Meriones unguiculatus*) macrophages by the filarial parasite *Brugia pahangi*. *Infect. and Immunit.* *43*:43-48, 1984.

Karavodin, L.M. and L.R. Ash. Circulating immune complexes in experimental filariasis. *Exp. Immunol.* *40*:312-317, 1980.

Karavodin, L.M. and L.R. Ash. Sequential determination of circulating immune complexes in experimental filariasis. *Infection & Immunity*. *34*:105-110, 1981.

Karavodin, L.M. and L.R. Ash. Inhibition of adherence and cytotoxicity by circulating immune complexes formed in experimental filariasis. *Parasite Immunol.* *4*:1-12, 1982.

Kayes, S.G. and D.G. Colley. Immunoregulation in experimental schistosomiasis. II. Soluble egg antigen-induced chronic spleen cell augmentation of baseline lymphocyte reactivity. *Cell Immunol.* *83*:152, 1984.

Kayes, S.G. and D.G. Colley. Immunoregulation in experimental schistosomiasis. III. Role of macrophages in soluble egg antigen-induced chronic spleen augmentation of baseline lymphocyte reactivity. *Cell. Immunol.* *83*:161, 1984.

Khoury, P.B. and S.M. Phillips. Kinetics and characterization of antigen-binding and antibody-producing cells in the regional draining lymph nodes and spleen during initial murine schistosomiasis. II. Cellular responses against egg antigens. *Cell. Immunol.* 59:246, 1981.

Klei, T.R. and W.A. Crowell. Pathological changes in kidneys, livers, and spleens of *Brugia pahangi*-infected jirds (*Meriones unguiculatus*). *J. Helminthol.* 55:123–131, 1981.

Klei, T.R., F.M. Enright, D.P. Blanchard, and S.A. Uhl. Specific hyporesponsive granulomatous tissue reactions in *Brugia pahangi*-infected jirds. *Acta Tropica 38*:267–276, 1981.

Klei, T.R., F.M. Enright, D.P. Blanchard, and S.A. Uhl. Effects of presensitization on the development of lymphatic lesions in *Brugia pahangi*-infected jirds. *Amer. J. Trop. Med. Hyg. 31*:280–291, 1982.

Klei, T.R., J.W. McCall, and J.B. Malone. Evidence for increased susceptibility of *Brugia pahangi*-infected jirds (*Meriones unguiculatus*) to subsequent homologous infections. *J. Helminthol. 54*:161–165, 1980.

Knight, D.H., R.B. Grieve, and L.T. Glickman. Measurement of antibody to *Dirofilaria immitis* as an indication of infection status following adulticide administration in dogs. *Proc. Heartworm Symp.* 1983. Veterinary Medicine Publishing Company, Edwardsville, Kansas, pp. 48–52, 1984.

Krulewitz, A., M.J. Stadecker, J.A. Wright, and B.L. Fanburg. Macrophage-associated angiotensin-I-converting enzyme activity in murine schistosomiasis. *Infect. and Immun. 41*:39, 1983.

Kuntz, R.E., T.C. Huang, and J.A. Moore. The capuchin monkey (*Cebus apella*) as an experimental host for *Schistosoma intercalatum*. *Proc. Helminthol. Soc. Wash. 47*:260–262, 1980.

Kuntz, R.E., J.A. Moore, and T.C. Huang. Variable *Schistosoma intercalatum* infection in cynomolgus macaques (*Macaca fascicularis*). *J. of Med. Primatol. 10*:175–179, 1981.

Kuntz, R.E., G.S. Tulloch, D. Van Riper, T.C. Huang, and J.A. Moore. *Schistosoma mansoni in vivo* exposure to hyperbaric oxygen. *J. Parasitol. 66*:364–365, 1980.

Lammie, P.J., S.M. Phillips, G.P. Linette, A.I. Michael, and A.G. Bentley. *In vitro* granuloma formation using defined antigenic nidi. *Proc. N.Y. Acad. Sci.* In press.

Landsperger, W.J., M.A. Stirewalt, and M.H. Dresden. Purification and properties of a proteolytic enzyme from *Schistosoma mansoni* cercariae. *Biochem. J. 201*:137, 1982.

Levinson, G. and T.J. Bradley. Removal of insect basal laminae using elastase. *Tissue and Cell 16*:367–376, 1984.

Lewert, R.M., M.G. Yogore, Jr., and B.L. Blas. Seroepidemiology of schistosomiasis japonica by ELISA in the Philippines. II. Unreliability of stool examination in the measurement of incidence. *Amer. J. Trop. Med. Hyg. 33*:872–881, 1984.

Lewert, R.M., M.D. Yogore, Jr., W.P. Martin, and B.L. Blas. Schistosomiasis japonica in Barrio San Antonio, Basey, Samar in the Philippines. IV. "Atypical" precipitates in the circumoval precipitin test—A reaction of recent infection. *Amer. J. Trop. Med. Hyg.* 29:431–434, 1980.

Lewis, F.A., S. Hieny, and A. Sher. Evidence against the existence of specific *Schistosoma mansoni* subpopulations which are resistant to irradiated vaccine induced immunity. *Amer. J. Trop. Med. Hyg.* In press.

Lewis, F.A. and M.A. Stirewalt. Effect of a cryopreserved live vaccine on resistance in mice with a pre-existing *Schistosoma mansoni* infection. *Amer. J. Trop. Med. Hyg.* In press.

Lewis, F.A. and E.M. Wilson. Strain differences in lymphocyte responses and *in vitro* suppressor cell induction between *Schistosoma mansoni*-infected C57Bl/6 and CBA mice. *Infect. and Immunol. 32*:260–267, 1981.

Lewis, F.A. and E.M. Wilson. Splenic lymphocyte responses of mice following an initial exposure to highly irradiated *Schistosoma mansoni* cercariae. *Exp. Parasitol. 54*:21–32, 1982.

Lewis, F.A. and E.M. Wilson. Regional and splenic lymphocyte proliferative responses of mice exposed to normal or irradiated *Schistosoma mansoni* cercariae. *Amer. J. Trop. Med. Hyg. 31*:505–513, 1982.

Libby, P., D.J. Wyler, M.W. Janicka, and C.A. Dinarello. Differential effects of human interleukin 1 on growth of human fibroblasts and vascular smooth muscle and endothelial cells. *Arteriosclerosis*. In press.

Little, III, J.V., C.E. Carter, and D.G. Colley. Serologic responses to *Schistosoma japonicum*: Evaluation of total and parasite-specific immunoglobulins during the course of murine infection. *J. Parasit. 68*:519, 1982.

Lok, J.B., M. Mika-Grieve, and R.B. Greve. Cryopreservation of *Dirofilaria immitis* microfilariae and third-stage larvae. *J. Heminthol. 57*:319–324, 1983.

Lok, J.B., M. Mika-Grieve, R.B. Grieve, and T.K. Chin. *In vitro* cultivation of third- and fourth-stage larval *Dirofilaria immitis*: Comparison of basal culture media, serum levels and possible serum substitutes. *Acta Tropica 41*:145–154, 1984.

Long, G.W., R.M. Lewert, and R.P. Pelley. Characterization of glycoprotein antigens which mediate the *Schistosoma japonicum* circumoval precipitin reaction. *Infect. Immun. 34*:389–396, 1981.

Long, G.W., R.M. Lewert, and R.P. Pelley. Fractionation of *Schistosoma japonicum* soluble egg antigen glycoproteins by hydrophobic interaction chromatography. *Infect. Immun. 34*:397–406, 1981.

Long, G.W., R.P. Pelley, B.L. Blas, M.G. Yogore, Jr., and R.M. Lewert. Analysis of the immunoglobulins responsible for the circumoval precipitation reaction. *Amer. J. Trop. Med. Hyg.* 29:1241–1245, 1980.

Long, G.W., M.G. Yogore, R.M. Lewert, B.L. Blas, and R.P. Pelley. Efficacy of purified *Schistosoma japonicum* egg antigens for ELISA serodiagnosis of human schistosomiasis japonica: Specificity and sensitivity. *Amer. J. Trop. Med. Hyg. 31*:1006–1014, 1982.

Loveless, S.E., S.R. Wellhausen, D.L. Boros, and G.H. Heppner. Tumoricidal macrophages isolated from liver granulomas of *Schistosoma mansoni*-infected mice. *J. Immunol. 128*:284, 1982.

LoVerde, P.T. Immunogenic characteristics of microbial components and products: Parasites. *In: Microbial Antigenodiagnosis*, K. Wicher, ed. *CRC Press, Inc.* In press.

LoVerde, P.T., H. van Keulen, L. Bobek, R. Gregoire, M. Simurad, S. Modavi, L. Spencer, and D. Rekosh. Role of protective antibodies in parasitic infections. *In: Antibodies: Protective, Destructive and Regulatory Role. 9th International Convocation on Immunology*, F. Milgrom, C.J. Abeyounis, eds. In press.

LoVerde, P.T. and R.E. Kuntz. Chromosome numbers of some schistosomes. *J. Parasitol. 67*:726, 1981.

Lowrie, R.C., Jr., T.C. Orihel, and M.L. Eberhard. *Culicoides variipennis*, a laboratory vector for the Amazon form of *Mansonella ozzardi*. *Amer. J. Trop. Med. Hyg. 31*:166–167, 1982.

McAllister, P.R., M.J. Dotson, S. Grim, and G.R. Hillman. Effects of phosphonium compounds on *Schistosoma mansoni*. *J. Med. Chem. 23*:862–865, 1980.

McCutchan, T.F., A.J.G. Simpson, J.A. Mullins, A. Sher, T.E. Nash, F.A. Lewis, and C. Richards. Differentiation of schistosomes by species, strain, and sex by using cloned DNA markers. *Proc. Nat. Acad. Sci. 81*:889–893, 1984.

Moore, J.A., R.E. Kuntz, and T.C. Huang. Exfoliative cytology in experimental infections with *Schistosoma haematobium*. *Acta Cytol. J. 24*:247–251, 1980.

Moser, G., F. von Lichtenberg, and A. Sher. Studies of the antibody-dependent killing of schistosomula of *S. mansoni* employing haptenic target antigens. III. Analysis of mechanisms responsible for the rejection of parasites *in vivo*. *Parasitology 83*:543–558, 1981.

Nagy, B.A., S.K. File, and J.H. Smith. Changes in the enteric vasculature of mice infected with *Schistosoma mansoni*. *Am. J. Trop. Med. Hyg. 30*:999–1009.

Nayar, J.K., J.C. Djan, and J.W. Knight. The susceptibility of *Anopheles quadrimaculatus* and other Florida mosquitoes to *Brugia patei* (Nematoda:Filarioidea). *Mosquito News 44*:417–419, 1984.

Nechay, B.R., G.R. Hillman, and M.J. Dotson. Properties and drug sensitivity of adenosine triphosphatases from *Schistosoma mansoni*. *J. Parasitol. 66*:596–600, 1980.

Neilson, J.T.M., C.A. Crandall, and R.B. Crandall. Adoptive and passive transfer of resistance to *Dipetalonema viteae* in hamsters. *Acta Tropica, 38*:309–318, 1981.

Nelson, P.D., D.J. Weiner, and B.E. Stromberg. Growth and molting of third stage *Litomosoides carinii in vitro*. *J. Parasitol. 68*:971–973, 1982.

Norden, A.P. and M. Strand. *Schistosoma*: Identification of genus-, species-, and gender-specific antigenic worm glycoproteins. *Exp. Parasitol. 57*:110–123, 1984.

[illegible] A.P. and M. Strand. *Schistosoma mansoni, S.* [illegible]*obium*, and *S. japonicum*: Identification of genus- and species-specific antigenic egg glycoproteins. *Exp. Parasitol. 58* 1984.

Norden, A.P. and M. Strand. Identification of antigenic *Schistosoma mansoni* glycoproteins during the course of infection in mice and humans. *Amer. J. Trop. Med. Hyg.* In press.

Pax, R.A., C.S. Bricker, D.P. Thompson, D.R. Semeyn, and J.L. Bennett. Neurophysiology of adult male *Schistosoma mansoni*. *Pharm. Ther. 22*:117–125, 1983.

Pax, R.A., C. Siefker, T. Hickox, and J.L. Bennett. *Schistosoma mansoni*: Neurotransmitters, longitudinal musculature and effects of electrical stimulation. *Exp. Parasitol. 52*:346–355, 1981.

Pax, R.A., D.P. Thompson, and J.L. Bennett. Action of praziquantel and the benzodiazepines on adult male *S. mansoni*. In: *Mechanisms of Drug Action*. Singer (ed.), *Academic Press*, New York. In press.

Phillips, S.M. and E.G. Fox. The immunopathology of parasitic disease. *Clin. Immunol. Allergy* 2:667, 1982.

Phillips, S.M. and E.G. Fox. The immunopathology of parasitic diseases: A conceptual approach. *In: Contemporary Topics in Immunobiology*, J.J. Marchalonis, ed. *Plenum Press*, New York. *12*:421, 1984.

Phillips, S.M., W.A. Reid, B.L. Doughty, and A.G. Bentley. The immunologic modulation of morbidity in schistosomiasis studies in athymic mice and *in vitro* granuloma formation. *Amer. J. Trop. Med. Hyg. 29*:820, 1980.

Popiel, I. and P.F. Basch. Putative transfer of polypeptides from male to female *Schistosoma mansoni*. *Mol. Biochem. Parasitol. 11*:179–188, 1984.

Popiel, I. and P.F. Basch. Reproductive development of female *Schistosoma mansoni* following bisexual pairing of worms and worm segments. *J. Exper. Zool.* In press.

Popiel, I., D. Irving, and P.F. Basch. Wound healing in *Schistosoma mansoni*. *Tissue and Cell.* In press.

Powell, M.R. and R.T. Damian. Micro-system for the primary immunization of baboon (*Papio cynocephalus*) peripheral blood mononuclear cells with sheep erythrocytes *in vitro*. *J. Immunol. Methods 48*:75–80, 1982.

Powell, M.R. and R.T. Damian. Schistosomiasis mansoni in baboons. VI. Plastic-adherent-cell suppression of the primary *in vitro* antibody response of peripheral blood mononuclear cells to a heterologous antigen (SRBC) during chronic infection. *Amer. J. Trop. Med. Hyg. 31*:790–795, 1982.

Powers, K.S., E.G. Platzer, and T.J. Bradley. The effect of nematode parasitism on the osmolality and major cation concentration in the hemolymph of three larval mosquito species. *J. Insect. Physiol. 30*:547–550, 1984.

Rand, T.H., J.A. Clanton, and D.G. Colley. Arachidonic acid metabolism in the murine eosinophil. III. Effect of nonsteroid anti-inflammatory drugs on lymphokine-directed eosinophil migration *in vivo*. *J. Immunol. 130*:1356, 1983.

Rand, T.H., J.A. Clanton, V. Runge, D. English, and D.G. Colley. Murine eosinophils labeled with Indium-111 oxine: Localization to delayed hypersensitivity reactions against a schistosomal antigen and to lymphokine *in vivo*. *Blood. 61*:732, 1983.

Rand, T.H. and D.G. Colley. Influence of a lymphokine fraction containing eosinophil stimulation promoter (ESP) on oxidative and degranulation responses of murine eosinophils. *Cell. Immunol. 71*:334, 1982.

Rand, T.H. and D.G. Colley. Lymphokine-mediated regulation of murine eosinophils. *In: Immunobiology of the Eosinophil.* T. Yoshida and M. Torisu, eds., Elsevier/North Holland, New York, p. 13, 1983.

Rand, T.H., J. Turk, R.L. Maas, and D.G. Colley. Arachidonic Acid metabolism in the Murine Eosinophil. II. Involvement of the lipoxygenase pathway in the response to the lymphokine eosinophil stimulation promoter. *J. Immunol. 129*:1239, 1982.

Robinson, A. and R.M. Lewert. Immunoglobulin M-Immunoglobulin G mixed cryoglobulinemia in *Schistosoma japonicum*-infected rabbits. *Infect. Immun. 39*:1477–1480, 1983.

Rosenwasser, L.J. and D.J. Wyler. Spontaneous immune reactivity to collagen in *Schistosoma mansoni* infected mice. *Amer. J. Trop. Med. Hyg.* In press.

Runge, V.M., T.H. Rand, J.A. Clanton, J.P. Jones, D.G. Colley, C.L. Partain, and A.E. James, Jr. Radionuclide imaging in In-111-labeled eosinophils in mice. *Radiology. 147*:563, 1983.

Sakaguchi, Y., I. Tada, L.R. Ash, and Y. Aoki. Chromosomes of two species of filarial worms, *Brugia pahangi* and *Brugia malayi* (Filariidae: Nematoda). *Chromosome Information Service*, No. 32, 12–14, 1982.

Sakaguchi, Y., I. Tada, L.R. Ash, and Y. Aoki. Karyotypes of *Brugia pahangi* and *Brugia malayi* (Nematoda: Filarioidea). *J. Parasitol. 69*:1090–1093, 1983.

Salama, M., W.S. Aronstein, J.B. Weiss, and M. Strand. Monoclonal antibody identification of protein antigens in livers of mice infected with *Schistosoma mansoni*. *Amer. J. Trop. Med. Hyg. 33*:608–620, 1984.

Samuelson, J.C. and J.P. Caulfield. Loss of covalently labeled glycoproteins and glycolipids from the surface of newly transformed schistosomula of *S. mansoni*. *J. Cell Biol. 94*:363–369, 1982.

Samuelson, J.C., J.P. Caulfield, and J.R. David. *Schistosoma mansoni*: Post transformational surface changes occurring in schistosomula grown *in vitro* and in mice. *Exp. Parasitol. 50*:369–83, 1980.

Samuelson, J.C., J.P. Caulfield, and J.R. David. Schistosomula of *S. mansoni* clear concanavalin A from their surface by sloughing. *J. Cell. Biol. 94*:355–362, 1982.

Samuelson, J.C., A. Sher, and J.P. Caulfield. Newly transformed schistosomula spontaneously lose surface antigens and C3 acceptor sites during culture. *J. Immunol. 124*:2055–57, 1980.

Samuelson, J.C., J.J. Quinn, and J.P. Caulfield. Hatching, chemokinesis, and transformation of miracidia of *Schistosoma mansoni*. *J. Parasitol.* In press.

Samuelson, J.P., J.J. Quinn, and J.P. Caulfield. Video microscopy of swimming and secreting cercariae of *Schistosoma mansoni*. *J. Parasitol.* In press.

Satmary, W.M. and T.J. Bradley. Dissociation of insect Malpighian tubules into single, viable cells. *Amer. Zool. 22*:913, 1982.

Satmary, W.M. and T.J. Bradley. The distribution of cell types in the Malpighian tubules of *Aedes taeniorhynchus* (Wiedeman) (Diptera: Culicidae). *Int. J. Insect. Morph. & Embryol. 13*:209–214, 1984.

Satmary, W.M. and T.J. Bradley. Dissociation of insect Malpighian tubules into single, viable cells. *J. Cell. Sci.* 1985. In press.

Sauerman, D.M., Jr. and J.K. Nayar. A survey of vectors of *Dirofilaria immitis* in Vero Beach, Florida. *Mosquito News 43*:222–225, 1983.

Sauerman, D.M. and J.K. Nayar. Characterization of refractoriness in *Aedes aegypti* to infection by *Dirofilaria immitis*. *J. Med. Entomol.* In press.

Sauerman, D.M. and J.K. Nayar. Prevalence of presumed *Dirofilaria tenuis* microfilariae in raccoons near Vero Beach, Florida. *J. Parasitol.* In press.

Schiller, E.L., R. D'Antonio, and H.M. Figueroa. Intradermal reactivity of excretory and secretory products of onchocercal microfilariae. *Amer. J. Trop. Med. Hyg.* *29*:1215–1219, 1980.

Schook, L.B., S.R. Wellhausen, D.L. Boros, and J.E. Niederhuber. Accessory cell function of liver granuloma macrophages of *Schistosoma mansoni*-infected mice. *Infect. Immun.* *42*:882, 1983.

Semeyn, D.R., R.A. Pax, and J.L. Bennett. Surface electrical activity from *Schistosoma mansoni*: A sensitive measure of drug action. *J. Parasitol.* *68*:353–363, 1982.

Siefker, C., R.A. Pax, and J.L. Bennett. *Schistosoma mansoni*: A comparison of male and female muscle physiology. *Comp. Biochem. Physiol.* *76C-2*:377–382, 1983.

Simpson, A.J.G., J.B. Dame, F.A. Lewis, and T.F. McCutchan. The arrangement of ribosomal RNA genes in *Schistosoma mansoni*. Identification of polymorphic structural variants. *Eur. J. Biochem.* *139*:41–45, 1984.

Sogandares-Bernal, F., R.E. Kuntz, M.V. Dennis, M. Voge, J.A. Moore, and T. Huang. Antigens in the Serum of *Macaca fascicularis* infected with tetrathyridia of *Mesocestoides corti*. *J. Parasitol.* *67*:591–592, 1981.

Srivastava, A.K. and J.J. Jaffe. Phosphatidylcholine synthesis in adult *Dirofilaria immitis* females. *Int. J. Parasitol.* In press.

Srivastava, A.K., J.J. Jaffe, and R.A. Lambert. Phosphatidylethanolamine synthesis in adult *Dirofilaria immitis* females. *Int. J. Parasitol.* In press.

Stadecker, M.J. The biology of mononuclear phagocytes. *In: Update in basic and applied immunology I. 1st Arg. Congress of Immunol.* In press.

Stadecker, M.J. Immunobiology of the granulomatous inflammation. *In: Update in basic and applied immunology I. 1st Arg. Congress of Immunol.* In press.

Stadecker, M.J. and S.A. Ebner. Characterization of mononuclear phagocytes in schistosomal egg granulomas of athymic mice. *J. Immunol.* *133*:2231, 1984.

Stadecker, M.J. and J.A. Wright. Distribution and kinetics of mononuclear phagocytes in granulomas elicited by eggs of Schistosoma mansoni. *Amer. J. Path.* *116*:245, 1984.

Stadecker, M.J., D.J. Wyler, and J.A. Wright. Ia antigen expression and antigen-presenting function by macrophages isolated from hypersensitivity granulomas. *J. Immunol.* *128*:2739–2744, 1982.

Stavitsky, A.B. and K.S. Garb. Spontaneous and egg antigen-induced syntheses of immunoglobulin and antibody by spleen cells and hepatic granulomas of mice infected with *Schistosoma japonicum*. *Infect. Immun.* *46*:276, 1984.

Stek, M., Jr., G.V. Hillyer, D. Cruess, and E.G. Hayunga. Immunization in murine schistosomiasis mansoni using soluble trematode antigens and irradiation attenuated vaccines. *Proc. Helm. Soc. Wash.* *51*:144–174, 1984.

Strand, M., W.S. Arsonstein, A.P. Norden, S.A. Lewis and J.B. Weiss. *Schistosoma worm proteins: Composition and Immunoreactivity.* The Third John Jacob Abel Symposium on Molecular Parasitology, J. Thomas August, ed., *Academic Press*, 1984.

Sunday, M.E., M.J. Stadecker, J.A. Wright, I. Aoki, and M.E. Dorf. Induction of immune responses by schistosome granuloma macrophages. *J. Immunol.* *130*:2413, 1983.

Sung, C.K. and M.H. Dresden. Assessment of viability of *Schistosoma mansoni* miracidia by congo red staining. *Amer. J. Trop. Med. Hyg.* In press.

Sutherland, D.R. and B.M. Christensen. Defense reactions of mosquitoes to filarial worms: Role of the microfilarial sheath in the response of mosquitoes to inoculated *Brugia pahangi* microfilariae. *J. Invert. Pathol.* In press.

Suzuki, T. and R.T. Damian. Schistosomiasis mansoni in baboons. IV. The development of antibodies to *Schistosoma mansoni* adult worm, egg, and cercarial antigens during acute and chronic infections. *Amer. J. Trop. Med. Hyg.* *30*:825–835, 1981.

Sweeny, T.L., P. Guptavanij, and A.R. Barr. Cytogenetic study of a sex-linked lethal in *Culex pipiens* L. In *Cytogenetics and Genetics of Vectors*: R. Pal, J.B. Kitzmiller, and T. Kanda, eds. Kodansha Ltd. Tokyo. pp. 147–159, 1981.

Tadano, T. Short-antenna, a new mutant in the mosquito *Aedes togoi*. *Jap. J. Sanit. Zool.* *33*:105–109, 1982.

Tadano, T. A sex-linked mutant, hooked leg, in the mosquito *Aedes (Finlaya) togoi*. *Mosquito News* *42*:72–75, 1982.

Tadano, T. Linkage studies on a new mutant, bleached pupa, in *Aedes (Finlaya) togoi*. *Mosquito News* *42*:61–65, 1982.

Tadano, T. Linkage studies on an esterase locus in the mosquito *Aedes togoi*. *Biochem. Genet.* *20*:711–721, 1982.

Tadano, T. Genetic linkage relationships between two enzyme loci, *Est-2* and *Acph*, in the mosquito *Aedes (Finlaya) togoi*. *Biochem. Genet.* *21*:477–485, 1983.

Tadano, T. Genetics of octanol and glycerophosphate dehydrogenases in the mosquito *Aedes (Finlaya) togoi*. *Biochem. Genet.* *21*:1167–1174, 1983.

Tadano, T. A genetic linkage map of the mosquito *Aedes togoi*. *Jap. J. Genet.* *59*:165–176, 1984.

Tarleton, R.L. and W.M. Kemp. Demonstration of IgG-Fc and C3 receptors on adult *Schistosoma mansoni*. *J. Immunol.* *126*:379-384, 1981.

Thompson, J.P., A.G. Bentley, R.B. Crandall, and C.A. Crandall. The histology and ultrastructure of the Meyrs-Kouwenaar body in ferrets infected with *Brugia malayi*. *Am. J. Trop. Med. Hyg.* In press.

Thompson, J.P., R.B. Crandall, C.A. Crandall, and J.T.M. Neilson. Microfilaremia and antibody responses in CBA/H and CBA/N mice following injection of microfilariae of *Brugia malayi*. *J. Parasit.* *67*:728-730, 1981.

Thompson, D.P., D.D. Morrison, R.A. Pax, and J.L. Bennett. Change in glucose metabolism and cyanide-sensitivity in *S. mansoni* during development. *Mol. Biochem. Parasitol.* *13*:39-51, 1984.

Thompson, D.P., R.A. Pax, and J.L. Bennett. Microelectrode studies of the tegument and subtegumental compartments of male *Schistosoma mansoni*. II. An analysis of electrophysiological properties. *Parasitology* *85*:163-178, 1982.

Thompson, D.P., R.A. Pax, and J.L. Bennett. *S. mansoni:* A comparative study of schistosomula transformed mechanically and by skin penetration. Electrophysiological responses to a wide range of substances. *Parasitology* *88*:477-489, 1984.

Trpis, M. Susceptibility of the autogenous group of the *Aedes scutellaris* complex of mosquitoes to infection with *Brugia malayi* and *Brugia pahangi*. *Tropenmed. Parasitol.*, *32*:184-188, 1981.

Trpis, M., R.E. Duhrkopf, and K.L. Parker. Non-Mendelian inheritance of mosquito vectors' susceptibility to infection with *Brugia malayi* and *Brugia pahangi*. *Science 211*:1435-1437, 1981.

Trpis, M., J.B. Perrone, M. Reissig, and K.L. Parker. Control of cytoplasmic incompatibility in the *Aedes scutellaris* complex by treatment of larvae with heat or antibiotics. *J. Heredity* *72*:313-317, 1981.

Turk, J., T.H. Rand, R.L. Maas, J.A. Lawson, A.R. Brash, L.J. Roberts, II, D.G. Colley, and J.A. Oates. Arachidonic acid metabolism in the murine eosinophil. Identification of lipoxygenase products from cells stimulated with ionophore A23187 or the lymphokine eosinophil stimulation promoter. *Biochem. Biophys. Acta.* *750*:78, 1983.

Vickery, A.C., A.L. Vincent, and W.A. Sodeman, Jr. Effect of immune reconstitution on resistance to *Brugia pahangi* in congenitally athymic nude mice. *J. Parasitol.* *69*:478-485, 1983.

Vincent, A.L., L.R. Ash, G.E. Rodrick, and W.A. Sodeman, Jr. The lymphatic pathology of *Brugia pahangi* in the Mongolian jird. *J. Parasitol.* *66*:613-620, 1980.

Vincent, A.L., J.W. McCall, L.M. Cowgill, L.R. Ash, and W.A. Sodeman, Jr. Infectivity of jird-passaged and dog-passaged strains of *Brugia pahangi* (Nematoda: Filarioidea). *J. Parasitol.* *68*:969-971, 1982.

Vincent, A.L. and A.C. Vickery. *Brugia pahangi:* Antibodies and microfilaremias in Lewis rats. *Exp. Parasitol.* *56*:381-390, 1983.

Vincent, A.L., A.C. Vickery, M.J. Lotz, and U. Desai. The lymphatic pathology of *Brugia pahangi* in nude (athymic) and thymic mice, C3H/HeN. *J. Parasitol.* *70*:48-56, 1984.

Vincent, A.L., A.C. Vickery, J.K. Nayar, D.M. Sauerman, and B.G. Yangco. Nondevelopment of *Wuchereria bancrofti* in nude (congenitally athymic) mice. *Am. J. Trop. Med. Hyg.* *31*:1062-1064, 1982.

Vincent, A.L., A.C. Vickery, A.W. Winters, and W.A. Sodeman, Jr. Life cycle of *Brugia pahangi* (Nematoda) in nude mice, C3H/HeN (nu/nu). *J. Parasitol.* *68*:553-560, 1982.

von Lichtenberg, F. Conference on contended issues of immunity to schistosomes. *Am. J. Trop. Med. Hyg.* 1984. In press.

von Lichtenberg, F. and J.E. Byram. Pulmonary cell reactions in resistance to *Schistosoma mansoni*. In: *The Host-Invader Interplay*, Van den Bossche, H. ed., Amsterdam, Elsevier/North Holland, pp. 405-416, 1980.

von Lichtenberg, F. and J.E. Byram. Pulmonary cell reactions in natural and acquired host resistance to *S. mansoni*. *Amer. J. Trop. Med. Hyg.* *29*:1286-1300, 1980.

von Lichtenberg, F. and J.E. Byram. Immunopathology of tissue invading trematodes and cestodes. *In: Parasites, Their World and Ours*. D.F. Mettrick and S.S. Desser, eds. *Elsevier Biomedical*, Amsterdam 96-108, 1982.

von Lichtenberg, F., R. de Oliveira, and A. Sher. The fate of challenge schistosomula in C/57/B1/6J mice vaccinated with gamma-radiated cercariae of *Schistosoma mansoni*. *Amer. J. Trop. Med. Hyg.* 1984. In press.

Weiner, D.J., D. Abraham, and R. D'Antonio. The effect of diethylcarbamazine on microfilariae of *Litomosoides carinii in vitro* and *in vivo*. *Z. fur Parasitenk.* In press.

Weiner, D.J., D. Abraham, and R. D'Antonio. Transfer of the ability of jirds *(Meriones unguiculatus)* infected with *Litomosoides carinii* to retard development of challenge larvae with cells and serum. *J. of Helminthol.* In press.

Weiner, D.J., G. Aguiree, and R. Dubielzig. Ectopic site filariid infection with immunologic follow-up of the host. *In: Proc. Heartworm Symp.* G. Otto, ed. *Vet. Med. Publ. Co.* Edwardsville, Kansas, pp. 51-54, 1981.

Weiner, D.J. and B.Y. Leeds. *Litomosoides carinii:* Retardation of growth and migration of challenge infections of jirds *(Meriones unguiculatus)*. *J. of Helminthol.* In press.

Weiner, D.J., B.Y. Leeds, and E.J.L. Soulsby. *Litomosoides carinii:* Effect of diethylcarbamazine on microfilaremias of

Mastomys natalensis harboring old infections, new infections and transfused microfilariae. *J. Parasitol.* *68*:1105–1109, 1982.

Weinstock, J.V. and D.L. Boros. Heterogeneity of the granulomatous response in the liver, colon, ileum and ileal Peyer's patches to schistosome eggs in murine schistosomiasis mansoni. *J. Immunol.* *127*:1906, 1981.

Weinstock, J.V. and D.L. Boros. Alteration of the granulomatous response in murine schistosomiasis by the chronic administration of Captopril, an inhibitor of angiotensin I converting enzyme. *Gastroenterology* *81*:953, 1981.

Weinstock, J.V. and D.L. Boros. Production of an angiotensin I converting enzyme by liver granuloma macrophages of *Schistosoma mansoni* infected mice. *Gastroenterology* 82:106, 1982.

Weinstock, J.V. and D.L. Boros. Alteration of granuloma angiotensin I-converting enzyme activity by regulatory T lymphocytes in murine schistosomiasis. *Infect. Immun.* *35*:465, 1982.

Weinstock, J.V. and D.L. Boros. Organ-dependent differences in composition and function observed in hepatic and intestinal granulomas isolated from mice with schistosomiasis mansoni. *J. Immunol.* *130*:148, 1983.

Weinstock, J.V. and D.L. Boros. Modulation of granulomatous hypersensitivity. VI. T lymphocyte subsets influence mast cell density in liver granulomas of *Schistosoma mansoni*-infected mice. *J. Immunol.* *131*:959, 1983.

Weinstock, J.V., D.L. Boros, and J.B. Gee. Enhanced granuloma angiotensin I. converting enzyme activity associated with modulation in murine schistosomiasis. *Gastroenterology* *81*:48, 1981.

Weinstock, J.V., S.W. Chensue, and D.L. Boros. Modulation of granulomatous hypersensitivity. V. Participation of histamine receptor positive and negative lymphocytes in the granulomatous response of *Schistosoma mansoni* infected mice. *J. Immunol.* *130*:423, 1983.

Weinstock, J.V., M.N. Ehrinpreis, D.L. Boros, and J.B. Gee. Effect of SQ 14225, an inhibitor of angiotensin I-converting enzyme, on the granulomatous response to *Schistosoma mansoni* eggs in mice. *J. Clin. Invest.* *67*:931, 1981.

Wellhausen, S.R. and D.L. Boros. Comparison of Fc, C3 receptors and Ia antigens on the inflammatory macrophage isolated from vigorous or immunomodulated liver granulomas of schistosome-infected mice. *J. Reticuloendothel. Soc.* *30*:191, 1981.

Wellhausen, S.R. and D.L. Boros. Atrophy of the thymic cortex in mice with granulomatous schistosomiasis mansoni. *Infect. Immun.* *35*:1063, 1982.

Wellhausen, S.R., S.W. Chensue, and D.L. Boros. Modulation of granulomatous hypersensitivity. Analysis by adoptive transfer of effector and suppressor T lymphocytes involved in granulomatous inflammation in murine schistosomiasis. *In Basic and Clinical Aspects of Granulomatous Diseases.* D.L. Boros and T. Yoshida, Eds. Elsevier/North Holland, New York, p. 219, 1980.

Willcockson, W.S., M.J. Dotson, P. McAllister, S.O. Grim, A.E. Ahmed, and G.R. Hillman. Effects of phosphonium and chloroethyl-acetylcholine compounds on schistosome and vertebrate acetylcholinesterase. *J. Comp. Biochem. Physiol.* *72C*:101–102, 1982.

Willcockson, W.S. and G.R. Hillman. Drug effect on the 5HT response of *Schistosoma mansoni*. *Comp. Biochem. Physiol.* *77*:199, 1984.

Willcockson, W.S., M. Khalid, A.E. Ahmed, and G.R. Hillman. Effects of acetylcholine mustard analogs on schistosome and vertebrate neuromuscular preparations. *J. Pharmacol. Exp. Ther.* *218*:330–336, 1981.

Wolde Mussie, E. and J.L. Bennett. Plasma emission spectrometric analysis of inorganic ions in *S. mansoni*. *J. Parasitol* *68*:48–52, 1982.

Wolde Mussie, E., J. VandeWaa, R.A. Pax, R. Fetterer, and J. Bennett. *Schistosoma mansoni*: Calcium efflux and effects of calcium-free media on responses of the adult male musculature to praziquantel and other agent inducing contraction. *Exp. Parasitol.* *53*:270–278, 1982.

Wright, J.D. and A.R. Barr. The ultrastructure and symbiotic relationships of *Wolbachia* of mosquitoes of the *Aedes scutellaris* group. *J. Ultrastruct. Res.*, *72*:52–64, 1980.

Wright, J.D. and A.R. Barr. *Wolbachia* and the normal and incompatible eggs of *Aedes polynesiensis* (Diptera:Culicidae). *J. Invert. Pathol.*, *38*:409–18, 1981.

Wyler, D.J. Regulation of fibroblast functions by products of schistosomal egg granulomas: Potential role in the pathogenesis of hepatic fibrosis. *In: Cytopathology of Parasitic Diseases.* Ciba Foundation Symposium 99 (New Series). (D. Evered and G.M. Collins, eds.) Pittman Publishing Co., London, pp. 190–204, 1983.

Wyler, D.J. and A.E. Postlethwaite. Fibroblast stimulation in schistosomiasis. IV. Isolated egg granulomas elaborate a fibroblast chemoattractant *in vitro*. *J. Immunol.* *130*:1371–1375, 1982.

Wyler, D.J. and L. Rosenwasser. Fibroblast stimulation in schistosomiasis. II. Functional and biochemical characteristics of granuloma-derived fibroblast stimulating factor. *J. Immunol.* *129*:1706–1710, 1982.

Wyler, D.J., M.J. Stadecker, C.A. Dinarello, and J. O'Dea. Fibroblast stimulation in schistosomiasis. V. Egg granuloma macrophages spontaneously secrete a fibroblast stimulating factor. *J. Immunol.* *132*:3142–3148, 1984.

Wyler, D.J. and J. Tracy. Direct and indirect effects of soluble extracts of *Schistosoma mansoni* eggs on fibroblast proliferation *in vitro*. *Infect. Immun.* *38*:103–108, 1982.

Yangco, B.G., A.L. Vincent, A.C. Vickery, J.K. Nayar, and D.M. Sauerman. An epidemiologic survey of filariasis among the refugees in south Florida. *Amer. J. Trop. Med. Hyg.* *33*:246–251, 1984.

Yates, J.A. and R.C. Lowrie, Jr. Development of *Yatesia hydrochoerus* (Yates and Jorgenson, 1983) to the infective stage in ixodid ticks. *Proc. Helminthol. Soc. Wash. 51:* In press.

Yates, J., R.C. Lowrie, Jr., and M.L. Eberhard. Development of *Tetrapetalonema llewellyni* to the infective stage in *Culicoides hollensis*. *J. Parasitol.* 68:293–296, 1982.

Yoe, M.R. The Great Neglected Diseases. *Johns Hopkins Magazine 32*:28–30, 1981.

Yogore, M.G., Jr., R.M. Lewert, and B.L. Blas. *Schistosomiasis japonica* in Barrio San Antonio, Basey, Samar in the Philippines. V. The enzyme-linked immunosorbent assay (ELISA) compared with quantitative stool examination and the circumoval precipitin (COP) test. *Amer. J. Trop. Med. Hyg.* *30*:1252–1262, 1981.

Yogore, M.G., Jr., R.M. Lewert, and B.L. Blas. Seroepidemiology of schistosomiasis japonica by ELISA in the Philippines. I. Underestimation by stool examination of the prevalence of infection in school children. *Amer. J. Trop. Med. Hyg.* *32*:1322–1334, 1983.

Yogore, M.G., Jr., R.M. Lewert, and B.L. Blas. Seroepidemiology of schistosomiasis japonica by ELISA in the Philippines. III. Selective mass chemotherapy with praziquantel in a control program. *Amer. J. Trop. Med. Hyg. 33*:882–890, 1984.

Yoshino, T.P. Comparison of concanavalin A-reactive determinants on hemocytes of two *Biomphalaria glabrata* snail stocks: Receptor binding and redistribution. *Devel. Comp. Immunol.* 5:229–240, 1981.

Yoshino, T.P. Concanavalin A-induced receptor redistribution on *Biomphalaria glabrata* hemocytes: Characterization of capping and patching responses. *J. Invert. Pathol. 38*:102–112, 1981.

Yoshino, T.P. Lectin-induced modulation of snail hemocyte surface determinants: Clearance of Con A-receptor complexes. *Devel. Comp. Immunol.* 6:451–461, 1982.

Yoshino, T.P. Surface antigens of *Biomphalaria glabrata* (Gastropoda) hemocytes: Occurrence of membrane-associated hemolymph-like factors antigenically related to snail hemoglobin. *J. Invert. Pathol. 41*:310–320, 1983.

Yoshino, T.P. Lectins and antibodies as molecular probes of molluscan hemocyte surface membranes. *Devel. Comp. Immunol.* 7:641–644, 1984.

Yoshino, T.P. and C.J. Bayne. Mimicry of snail host antigens by miracidia and primary sporocysts of *Schistosoma mansoni*. *Parasite Immunol.* 5:317–328, 1983.

Yoshino, T.P. and C.D. Davis. Surface antigens of *Biomphalaria glabrata* (Gastropoda) hemocytes. Evidence for linkage-independence of some hemolymph-like surface antigens and Con A receptor-bearing macromolecules. *J. Invert. Pathol.* *42*:8–16, 1983.

Yoshino, T.P. and W.O. Granath, Jr. Identification of antigenically distinct hemocyte subpopulations in *Biomphalaria glabrata* (Gastropoda) using monoclonal antibodies to surface membrane markers. *Cell. Tiss. Res. 232*:553–564, 1983.

Joint Scientific Conferences

PARASITIC DISEASES PANEL

Date: July 29-August 2, 1980

Place: Tsukuba, Japan

Summary: The 15th Joint Conference of the Panels on Parasitic Diseases was attended by approximately 100 participants, 11 of them from the United States. Thirty-five papers were presented on various aspects of schistosomiasis and filariasis, including two mini-symposia on the topics of protective immunity in schistosome infections and onchocerciasis research and control in Latin America. A highlight of the meeting was the participation by two representatives from the People's Republic of China, Dr. Zhong Huei-Lan and Dr. Ho Lian-Yin. Dr. Zhong, Director of the Beijing Tropical Medicine Research Institute, summarized the present situation of filariasis in China and also showed a film on the control and chemotherapy of filariasis in his country.

Date: July 13-15, 1981

Place: Bethesda, Maryland

Summary: More than 50 participants, 9 of them from Japan, participated in the 16th Joint Conference of the Panels on Parasitic Diseases. A total of 38 papers in the program areas of schistosomiasis and filariasis were presented. A highlight of the meeting was a mini-symposium on schistosome chemotherapy. Eight investigators summarized the progress that is being made in the development of new antischistosomal compounds which may be effective against more than one species of schistosome and can be administered in a highly desirable dosage regimen.

Date: August 22-25, 1982

Place: Kumamoto, Japan

Summary: The 27th Joint Conference of the Panels on Parasitic Diseases was attended by more than 70 participants, including 7 scientists from the United States. A total of 34 papers on various aspects of schistosomiasis and filariasis research were presented, including a series of papers on various aspects of onchocerciasis research in Guatemala relating to the immunodiagnosis of infection, control, chemotherapy, and the identification of immune complexes and their role in humoral and cell-mediated suppression.

Date: July 18-19, 1983

Place: Bethesda, Maryland

Summary: Rather than the traditional meeting of the Joint Panels on Parasitic Diseases the Joint Committee of the U.S.-Japan Cooperative Medical Science Program and the Microbiology and Infectious Diseases Program of the National Institute of Allergy and Infectious Diseases of the National Institutes of Health sponsored a parasitic diseases Symposium on Current and Unresolved Problems in Parasitic Diseases. Approximately 40 invited participants, of equal numbers from Japan and the United States, as well as numerous parasitologists and tropical medicine specialists from the Washington area attended the conference. Dr. Manabu Sasa, a member of the Japanese Delegation and the first Chairman of the Japanese Panel on Parasitic Diseases, presented the keynote address on "Tropical parasitic diseases: their importance as public health problems in tropical countries, and the need for basic studies in their control and treatment." Topics presented covered many of the current tropical disease problems existent in the world including leishmaniases, American and African trypanosomiases, giardiasis, amebiasis, and parasites associated with the acquired immune deficiency syndrome (AIDS) — *Pneumocystis carinii*, *Cryptosporidium* and *Strongyloides stercoralis*. Dr. H. Hirumi of the International Laboratory for Research on Animal Diseases in Nairobi, Kenya, summarized his work on the *in vitro* cultivation of African trypanosomes. There were summary presentations on schistosomiasis and filariasis.

Date: August 21-24, 1984

Place: Tokyo, Japan

Summary: More than 100 participants, 10 of them from the United States, attended the 19th Joint Conference of the Panels on Parasitic Diseases. Thirty-six papers were presented on various aspects of filariasis, schistosomiasis, malaria, and vector biology. One afternoon session was devoted to a major symposium on the biology and control of vectors of lymphatic filariasis, onchocerciasis, malaria, and schistosomiasis.

TUBERCULOSIS PANEL

Drs. Tohru Tokunaga and Mayer Goren, Chairmen respectively of the Tuberculosis Panel, U.S.-Japan Cooperative Medical Science Program

Dr. Tohru Tokunaga, Chairman (1981– ,
Member 1978–1980)
Director
Department of Cellular Immunology
National Institute of Health
2-10-35, Kamiosaki, Shinagawa-ku
Tokyo 141, Japan

Dr. Mayer Goren, Chairman (1980–
Member 1977–1980)
Senior Research Scientist
Division of Research
National Jewish Hospital
Denver, Colorado 80206

PANEL MEMBERS

Japan

Dr. Ichiro Azuma (1981–)
Professor
Department of Biological Chemistry
Institute of Immunological Science
Hokkaido University
Kita 15, Nishi 7, Kita-ku
Sapporo 060, Japan

Dr. Kikuo Nomoto (1978–)
Professor
Department of Immunology
Institute of Bioregulation Research
Kyushu University
3-1-1, Maidashi, Higashi-ku
Fukuoka 812, Japan

Dr. Kazuhisa Saito (1978–)
Professor
Department of Microbiology
Keio University
School of Medicine
35, Shinano-machi, Shinjuku-ku
Tokyo 160, Japan

United States

Dr. Frank M. Collins (1980–)
Member
Trudeau Institute
P.O. Box 59
Saranac Lake, New York 12983

Dr. Jerrold J. Ellner (1983–)
Associate Professor
School of Medicine
Case Western Reserve University
2058 Abington Road
Cleveland, Ohio 44106

Dr. Maurice J. Lefford (1983–)
Wayne State University School of Medicine
Gordon H. Scott Hall of Basic Medical Science
Department of Immunology and Microbiology
540 East Canfield Avenue
Detroit, Michigan 48201

Dr. Kuni Takayama
Veterans Administration Hospital
2500 Overlook Terrace
Madison, Wisconsin 53705

Dr. Ichiro Toida (1978-)
Director
First Department of Research
Research Institute of Tuberculosis
Japan Anti-Tuberculosis Association
3-1-24, Matsuyama, Kiyose-shi
Tokyo 204, Japan

Former Member

Dr. Yuichi Yamamura, Chairman
(1978-1981, Member 1976-1977)
President
Osaka University
1-1, Yamadagaoka, Suita-shi
Osaka 565, Japan

Former Members

Dr. John R. David, Chairman (1977-1980,
Member 1975-1977)
Professor
Department of Medicine
Robert B. Brigham Hospital
Harvard University
Boston, Massachusetts 02120

Dr. George Mackaness (1977-1980)
President
Squibb Institute for Medical Research
Princeton, New Jersey 08540

Dr. Quentin N. Myrvik (1977-1982)
Professor and Chairman
Department of Microbiology and Immunology
Wake Forest University
Winston-Salem, North Carolina 27102

Dr. Takeshi Yoshida (1983-1984), Resigned
Professor
Department of Pathology
University of Connecticut Health Center
Farmington, Connecticut 06032

Japanese Tuberculosis Panel

BROAD GOALS

The basic character of the Tuberculosis Panel and guidelines for its activities were discussed and accepted at the Joint Panel Meeting in October 1965 at the start of the U.S.-Japan Cooperative Medical Science Program. Since then, studies on the mechanism of immunity and allergy in tuberculosis have been the key subjects of research activity of the panel. The scope of the panel's research activities was expanded during these 20 years and is summarized as follows:

- Biology of mycobacteria
- Purification and properties of biologically active constituents of mycobacteria
- Pathogenesis of tuberculosis and atypical mycobacteriosis
- Studies on the mechanism of immunity in tuberculosis
- Studies on the mechanism of delayed-type hypersensitivity in tuberculosis
- Immunogenetical studies on the immunity and allergy in tuberculosis
- Studies on the influences of the constituents of mycobacteria on the diseases other than tuberculosis
- Basic studies in cellular immunology in relation to tuberculosis

These studies were conducted under close collaboration with members of the United States and Japanese Tuberculosis Panels.

RESEARCH ACTIVITIES DURING THESE FIVE YEARS

Biology of Mycobacteria

Element analyses of the fine structure of mycobacteria were performed with the analytical electron microscope. Electron dense granules were found to contain high concentrations of phosphorus and potassium. Spheroplast formation and reversion to the bacillary form of mycobacteria were observed with electron microscope.

Resistance mechanisms to anti-tuberculosis drugs of mycobacteria, especially resistance due to enzymatic inactivation of drugs, were studied. Isoniazid-hydrolytic enzyme of *Mycobacterium smegmatis* and inactivating enzyme(s) of *M. fortuitum* for aminoglycoside- and peptide-antibiotics were reported.

Chemical Constituents of Mycobacteria and Their Biological Activities

Efforts to purify tuberculin active antigens continued. Routine procedures for protein purification, such as ammonium sulfate factionation, gel filtration, ion exchange chromatography and high performance liquid chromatography, were not effective in producing homogeneous tuberculin preparations with higher specific activity than PPD.

Protein antigens, designated as MPB-70 and -80 respectively, were found in culture filtrates of *M. bovis*, but not in culture filtrates of *M. tuberculosis*. These antigens elicited delayed-type skin reaction by guinea pigs sensitized with *M. bovis* but not by animals sensitized with *M. tuberculosis*. These results suggest the possibility that a specific tuberculin antigen could be used to differentiate BCG-sensitized people from those infected with human tubercle bacilli.

A new glycolipid, which existed in strain $H_{37}Rv$, a virulent strain of *M. tuberculosis*, but not in an avirulent $H_{37}Ra$ strain, was found and purified to homogeneity. The chemical structure was determined as polyacylated trehalose. This glycolipid was devoid of lethal toxicity to mice.

The molecular distribution of mycolic acids, high molecular alpha-branched-beta-hydroxy fatty acids characteristic for mycobacteria, nocardia and corynebacteria, was analyzed with GC-MS. Each bacterial species showed a characteristic distribution pattern for mycolic acid molecules. Furthermore, the molecular distribution of mycolic acids changed according to the growth temperature of bacilli in a characteristic way for respective bacilli; some bacterial species increased the proportion of longer chain molecular species and others increased the proportion of more unsaturated molecular species when grown at lower tempertures.

Pathogenesis of Tuberculosis

Pathogenesis of tuberculosis was investigated at cellular and subcellular levels. Electron microscopic observation of the lung of mice infected with virulent tubercle bacilli revealed that tubercle bacilli phagocytosed by macrophages existed within phagosomes in close contact with the phagosomal membrane. Such interaction between phagosomal membrane and tubercle bacilli activated phospholipase of phagocyte and/or tubercle bacilli and induced the damage of phagosomal membranes and the liberation of free fatty acids. These events might have significant meaning for the outcome of tuberculosis infections. To clarify the mode of interaction between phagosomal membrane and tubercle bacilli, analyses with artificial liposome were also carried out.

Immunological studies were continued to clarify the mechanism of granuloma formation which is the most characteristic pathohistological feature of tuberculosis. Congenitally athymic nude mice, when infected with avirulent BCG, allowed abundant proliferation of the bacilli within tissues, but granuloma formation was very limited. Passive transfer of sensitized T-lymphocytes from normal syngenic mice to such BCG-infected nude mice enhanced granuloma formation. These effective T-lymphocytes were Lyt $1^+ 2^-$ cells and their H-2 I region should be compatible with that of the recipient animal. As for the granuloma formation induced by the intravenous injection of BCG-CWS or MDP plus PPD, inbred strains of mice were classified into high- and low-responder strains. Mechanism of low responsiveness was found to be due to the low ability in antigen presentation of the macrophages of low responder mice. Strain difference in granuloma formation was parallel with the strain difference in ability to acquire immunity against tuberculosis infection. But strain difference did not correlate with the degree of development of delayed-type hypersensitivity.

The immunological mechanism for cavity formation was analyzed by injecting lymphokine together with BCG-CWS directly into the rabbit lung. The lymphokine induced the accumulation and activation of macrophages within lung tissue, and lysosomal enzymes released from such activated macrophages caused necrosis and liquefaction of surrounding tissue. A proteolytic enzyme which was responsible for such tissue destruction was partially purified and characterized enzymologically. The apo-protein portion of a lipoprotein isolated from mycobacteria, presumably the antigen for cavity formation, required an adjuvant such as B_{30}–MDP, to induce cavity formation.

Mechanism of Immunity in Tuberculosis

The immune mechanism in tuberculosis can be schematically outlined as follows:

- Phagocytosis and digestion of tubercle bacilli by macrophages
- Antigen presentation by macrophages to T-lymphocytes
- Sensitization of T-lymphocytes
- Lymphokine production by sensitized T-lymphocytes in reaction with the specific antigen
- Mobilization and activation of macrophages by lymphokine
- Enhanced phagocytosis and killing of tubercle bacilli by activated macrophages.

The respective roles of the immune competent cells and the interaction between these cells were analyzed in detail.

Role of macrophages and their activation

Neonatal thymectomy had little influence in immunity against *Listeria* infection, but seriously decreased the immunity against infection with tubercle bacilli. In the case of *Listeria* infection, the so-called "primitive T-cell response" had a significant role in protecting the host from the infection. For immunity against tuberculosis infection, much more potent activation of macrophages was required.

Macrophages can be activated by various types of stimulants. In case of activation of MDP, the cooperation of T-lymphocytes was not required. Biochemical mechanism of macrophage activation was studied in relation to arachidonic acid metabolism of macrophages. It was clarified that calcium-ionophore, which has the ability to activate macrophages, and formyl-methionyl-leucyl-phenylalanine, which has chemotactic ability for macrophages, stimulated different pathways of phospholipid metabolism, respectively.

Using monoclonal antibody techniques for the analyses of surface markers, macrophages, which developed according to their origin, stage of differentiation, or degree of activation, were carried out. Resident peritoneal macrophages, those induced with pyran, corynebacteria, thioglycolate or peptone, and alveolar macrophages showed different patterns of surface antigens. Asialo GM_1, a marker of activated macrophages, was detected only on the corynebacteria-induced cells among peritoneal macrophages, but it was always detected on alveolar macrophages, even on the resident ones. As markers for differentiation, 5'-nucleotidase, leucine-aminopeptidase, and alkaline phosphatase were also useful. The receptor for the activating factor for macrophages of mice was shown to contain D-mannose.

The steps of maturation from precursor cells to mature macrophages were analyzed with established cell line, M_1 cells, that originated from mouse myelitic leukemia cell. When development of Fc receptor and phagocytosis activity were used as markers of matured macrophages, lymphokine complex obtained by the stimulation with Con A or specific antigen induced the maturation of M_1 cells. Purified MIF/MAF had no such activity. Purified MIF/MAF induced matured macrophages to produce maturation (differentiation) enhancing factor for M_1 cells. Thus purified MIF/MAF could indirectly stimulate the maturation (differentiation) of immature macrophages.

In relation to human pulmonary diseases, the function of macrophages obtained from bronchoalveolar lavages was compared. When the development of IgG-Fc receptor, rosette-formation mediated by C_3- receptor and phagocytosis activity were used as markers of activation, macrophages obtained from healthy people or from healthy lungs of patients showed very low activities. Those obtained from lungs of persons suffering from tuberculosis, berillium lung, or sarcoidosis showed remarkedly enhanced activities.

Antigen presentation by macrophages

At the induction-phase of immunity, macrophages present antigenic stimulation to lymphocytes. To analyze the antigen-presentation activity of macrophages in detail, cell-lines of antigen-presenting cells (accessory cells) were established. Three lines of such cells were obtained by transforming guinea pig peritoneal macrophages or bone marrow cells with SV_{40} or EB virus. These established cell lines were all adherent and phagocytic, but were different from ordinary peritoneal macrophages in that they lacked Fc- and C_3-receptors.

Antigen-presenting activity of macrophages was examined by the lymphocyte-proliferation reaction with TNP as antigen. Spleen adherent cells showed the highest and peritoneal macrophages showed moderate activity. Alveolar macrophages not only had little activity but also suppressed the antigen-presenting activity of spleen adherent cells.

T-lymphocytes

Studies on T-lymphocyte are summarized in the next section.

Mechanism of Delayed-Type Hypersensitivity

Studies with cloned T-lymphocyte cell line

A colony of PPD-specific T-lymphocytes, which was formed in soft agar from lymph-node cells of PPD-sensitized mouse (BALB/c), was expanded in the presence of T-cell growth factor (TCGF). A PPD-specific T-lymphocyte clone (BP-3) was established by subculturing these cells in the presence of PPD and antigen-presenting cells. This cloned T-lymphocyte required I–A compatible antigen-presenting cells to react with PPD and did not produce TCGF by the reaction with PPD. When these cloned T-lymphocytes were injected into foot pads of BALB/c mice together with PPD, delayed-type foot pad swelling developed. Histological features of the foot pad reaction revealed remarkable thickening of dermis and abundant infiltration of mononuclear cells. This was in accord with those of typical delayed-type hypersensitive reactions. When cloned T-lymphocyte BP-3 cells and PPD were added to the monolayer culture of macrophages infected with *Salmonella enteritidis*, multiplication of *Salmonella* within macrophages was remarkably suppressed. BP-3 cells and PPD also inhibited intracellular multiplication of BCG. These results clearly demonstrated that the T-lymphocyte responsible for delayed-type hypersensitive reaction was identical with that responsible for cellular immunity to protect against infection.

The helper function for antibody synthesis of PPD-specific T-lymphocytes was analyzed with established PPD-specific cloned T-lymphocytes. Three types of clones were obtained, namely, clones which acted only in cognante interaction between B- and T-lymphocytes, those which acted only as helper T-lymphocytes by producing factor(s), and those which had both functions. All three types of clones produced T-lymphocyte growth factor in their culture supernatants when cultured with PPD and antigen-presenting cells. Only the latter two types of clones could produce B-lymphocyte growth factor and B-lymphocyte differentiation factor. Furthermore, cloned T-lymphocytes produced T-lymphocyte growth factor and B lymphocyte differentiation factor very strongly by the stimulation either with PPD and antigen-presenting cells or with Con A. Con A stimulation induced weaker production of B-lymphocyte growth factor than PPD stimulation.

Dynamics of T-lymphocytes in human tuberculosis

In one study PPD-specific T-lymphocytes were eliminated from peripheral blood lymphocytes from a tuberculosis patient by 5-deoxyuridine treatment, PPD-stimulation and light irradiation. The remaining cell population was added with X-irradiated peripheral blood lymphocytes from the same patient. ^{3}H-thymidine uptake and the number of autologous rosette forming T-lymphocytes were increased by stimulation with PPD. These results suggested the occurrence of T-lymphocyte transstimulation. Essentially the same phenomenon was observed in mice.

T-lymphocytes of tuberculosis patients were analyzed with monoclonal antibodies of the OKT series. Lymphocytes in pleural effluent of tuberculosis pleurisy patients were mostly T-lymphocytes of the OKT-4 cell (inducer/helper) type, by contrast, lymphocytes in peripheral blood of the same patients had a significantly higher proportion of OKT-8 cells (supressor/killer) than those of the healthy human control and those of tuberculosis patients without the complication of pleurisy. By stimulating with PPD, lymphocytes from pleural effluent increased OKT-4 cells, but those from peripheral blood increased OKT-8 cells.

PPD-reactive T-lymphocytes were demonstrated in cord blood of newborn babies from tuberculin-positive mothers. This suggests the possibility of passive transfer of cellular immunity from mother to baby.

When lymphocytes from tuberculosis patients were stimulated *in vitro* with PPD or a nonspecific mitogen, such as Con A and PHA, the number of Tac-positive cells increased. The peak was on the 6th day in the case of PPD and the 2nd day with Con A and PHA. The increase in the number of Tac-positive cells paralleled the increase of ^{3}H-thymidine uptake. Hydrocortisone did not influence these reactions, but cyclosporin A suppressed them. Lymphocytes stimulated with PPD in the presence of hydrocortisone absorbed IL-2. Those stimulated with PPD in the the presence of cyclosporin A did not. Furthermore, PPD-stimulation in the presence of IL-2 and hydrocortisone induced proliferation of lymphocytes; PPD-stimulation in the presence of IL-2 and cyclosporin A did not induce proliferation. These results showed that hydrocortisone suppressed the development of IL-2 receptor on T-lymphocytes. When Ia antigen positive, IL-2 dependent T-lymphocytes combined with PPD were used as the antigen-presenting cells, the number of Tac-positive cells of tuberculosis patients increased. They did not show any proliferation. These results indicated that PPD and Ia stimulation were sufficient to induce Tac-antigen on PPD-specific T-lymphocytes, but IL-1 and IL-2 were not required.

Suppressor-macrophages

When mice were previously injected intraveneously with large amounts of killed BCG, development of delayed-type hypersensitive reaction with PPD was suppressed. Such suppression was due to induction of suppressor cells, and was antigen-nonspecific and not restricted by the H-2 complex. This suppression was dependent on macrophages, and abolished by the treatment with indomethacin. Such suppressor cells resided originally in bone marrow and were released into peripheral tissues by the intravenous injection of large amounts of killed BCG.

Purification of lymphokine and its mode of action

Among the various lymphokines produced by PPD-reactive T-lymphocytes in reaction with PPD, macrophage migration inhibitory factor (MIF) was purified by affinity chromatography prepared with anti-MIF antibody. MIF obtained from guinea pig was shown to be a protein with a molecular weight of about 35,000. Its pI was 5.0–5.5.

The activities of MIF and MAF on macrophages were not augmented by addition of cAMP and cGMP. They were enhanced by the addition of calcium ionophore.

Tissue distribution of cells responsible for transferring PPD sensitivity, MIF-producing cells and LIF-producing cells, differed when these cells were passively transferred to sensitized animals. The result indicated that these functions were expressed by distinct cell populations.

Adjuvant active substances

Adjuvant activity of tubercle bacilli to induce delayed-type hypersensitivity was found to be localized in the cell wall of bacilli. It was found that the minimal structural unit for adjuvant activity was muramyl-L-alanyl-D-isoglutamine. Various structural analogues were chemically synthesized and the relation between their chemical structure and adjuvant and antitumor activities were compared. Potent adjuvant activity occurred in the MDP analog where L-alanine was replaced with L-valine and in MDP derivatives when 6-OH of muramic acid was substituted with the acyl- or quinonyl-group. MDP and its analogues had direct macrophage activating activity, anti-tumor activity, and arthritis-inducing activity. These activities were compared in relation to their adjuvant activity.

Immunogenetical Studies on the Immunity and Allergy in Tuberculosis

Genetic control of the responsiveness for the delayed-type hypersensitivity was analyzed with various inbred strains of mice. F_1 animals between high-responder and low-responder strain of mice were sensitized with BCG. Their T-lymphocytes were stimulated *in vitro* with PPD. When macrophages obtained from high-responder were added to the culture, ^3H-thymidine uptake of T-lymphocytes increased significantly. Macrophages from low-responder could not enhance the uptake. This suggested that the mechanism of low-responsiveness was due to the low antigen-presenting ability of macrophages of low-responder mice. Further, PPD-specific suppressor T-lymphocytes were induced *in vitro* when spleen cells of low-responder mice were cultured with PPD in the presence of I-J^k positive adherent cells. Such induction of suppressor cells was much more significant in low-responder than in high-responder mice. Similar strain-differences were observed in the delayed-type hypersensitive reaction with methylated human serum albumin as the antigen.

Strain differences were also observed in granuloma-formation induced by intravenous injection of mycoloyl-MDP + PPD or of BCG-CWS in mice. Inbred strains of mice were classified into high- and low-responder strains. Strain difference in the granuloma-formation correlated with the strain difference in the acquired resistance against tuberculosis infection. They did not correlate with the degree of delayed-type hypersensitive reactions.

Studies on the Influences of Chemical Constituents of Tubercle Bacilli on the Diseases Other Than Tuberculosis

The mechanism of anti-tumor activity of BCG or bacterial constituents remained unclear. Purified cord factor from BCG showed strong anti-tumor activity when used in combination with lipopolysaccharide (LPS) isolated from Salmonella. Efforts to reduce the toxicity of LPS were continued.

Mechanisms of low-responsiveness to induce killer T-lymphocytes against syngeneic tumor cells were evaluated. It was found that the induction of killer T-lymphocytes was restricted by H-2 D genes. Low-responder animals induced alternative effector cells other than killer T-lymphocytes by the repeated immunization with target cells. The anti-tumor activity of BCG-CWS was due to augumentation of the latter non-specific effector mechanism.

DNA preparation isolated from tubercle bacilli showed potent anti-tumor activity. 6-0-quinonyl derivative of MDP showed anti-tumor activity.

ACHIEVEMENT OF TUBERCULOSIS PANEL DURING THESE 20 YEARS

Biology of tubercle bacilli, biochemical studies on chemical constituents of mycobacteria, and studies on biological activities of chemical constituents:

- The chemical structure of cell walls of tubercle bacilli was shown to consist of glycolipid and peptidoglycan portion. The former is arabinogalactan polysaccaride esterified with mycolic acid and the latter is N-acetylglucosaminyl-N-glycollyl-muramyl-tetrapeptide.
- Very strong adjuvant activity of tubercle bacilli is localized in their cell wall. The minimal structural unit for the adjuvant activity is muramyl-dipeptide (MDP).
- Structural analogues of MDP were chemically synthesized and their adjuvant activity and anti-tumor activity examined in relation to their chemical structures. These findings suggest the possible use of some MDP-analogues as immunomodulators.
- By the introduction of GC-MS, the detailed structure of mycolic acids, high molecular fatty acids characteristic for mycobacteria, was established. Furthermore, it was shown that a species of mycobacteria contained heterologous molecular species of mycolic acids. The distribution of mycolic acids appears to be a characteristic of mycobacteria species, especially when grown at different temperatures.

Studies on pathogenesis of tuberculosis: The mechanism of granuloma formation was analyzed immunologically. Passive transfer experiments with nude mice showed that T-lymphocytes of Lyt $1^+ 2^-$ were important for granuloma formation. The H-2 I region of recipient animals and their T-lymphocytes should be compatible to enhance granuloma formation. Strain differences in granuloma formation appeared to be determined genetically.

Studies on the mechanism of immunity and allergy in tuberculosis: Twenty years ago little was known about mechanism of immunity and allergy in tuberculosis. It was thought that immunity to tuberculosis did not depend upon antibodies against tubercle bacilli but rather to an unknown cellular mechanism. Macrophages were considered to play important roles in tuberculosis immunity. Amazing progress on immunologic mechanisms during the last 20 years has considerably clarified the mechanism of immunity and allergy in tuberculosis. See "Mechanism of Immunity in Tuberculosis," above. Such significant progress in the modern immunologic concepts of tuberculosis accrued from work on cellular and hypersensitivity reactions. Members of the Tuberculosis Panel contributed importantly to this progress.

Roles of T-lymphocytes, classification of T-lymphocytes into subsets and their respective roles

The most remarkable achievement in immunology during the past 20 years is the clarification of the role of T-lymphocytes in immunity and understanding the presence of several subsets of T-lymphocytes with differing functions. Recently, it was possible to establish cloned T-lymphocyte cell-lines and analyze the respective roles of each subset. It was evident that T-lymphocytes played major roles in the development of delayed-type hypersensitivity. Those involved in protective immunity against tubercle bacilli belonged to identical cell populations. Thus, a long-conflicting subject on the relationship between immunity and allergy in tuberculosis was conclusively resolved.

Mechanism of the Activation of Macrophages and the Classification of Macrophages According to Their Surface Markers

Activated macrophages showed enhanced phagocytic and bacteriocidal activities. They were shown to play very important roles in defense mechanisms of the host against infection. Mechanisms of the activation of macrophages, especially activation by MDP or lymphokines, were clarified. Further, it was shown that macrophages expressed different surface markers according to their origin, and it became possible to differentiate macrophages according to their surface markers. The significance and role of Ia-antigen on macrophages was studied in detail.

MIF/MAF were highly purified by affinity column chromatography with an antibody preparation against MIF.

Immunogenetical Studies in Immunity and Allergy in Tuberculosis

Various inbred strains of mice were sensitized with tubercle bacilli. It was found that the high and low responsiveness to the delayed sensitivity reaction was genetically controlled. The mechanism of low-responsiveness results from the low antigen-presenting ability of macrophages of low-responders. Advances were made in clarifying genetic controls of granuloma-formation and natural resistance against the infection with tubercle bacilli.

Progress in tuberculosis research during 20 years of the U.S.-Japan Cooperative Medical Science Program relates directly to work of the Tuberculosis Panel Members and the willing cooperation between the U.S. and Japanese participants.

The knowledge obtained from fundamental studies in experimental animals, and the dynamic evaluation of human T-lymphocytes and macrophages in human mycobacterial and atypical mycobacterial infection, now open the way for extensive study. Such analyses should provide useful information for better diagnosis and treatment. Further progress in immunogenetics will enable better methods to determine susceptibility among risk-groups for tuberculosis or atypical mycobacteriosis.

FUTURE OBJECTIVES

- To characterize those factor(s) of tubercle bacilli which determine host pathogenicity;
- To purify to homogeneity a specific tuberculin antigen which will differentiate *M. tuberculosis* from *M. bovis* and other atypical mycobacteria;
- To develop a sensitive and specific serological test with appropriate antigens for the differential diagnosis of tuberculosis;
- To clarify mechanisms of resistance of mycobacteria to antituberculosis drugs and to develop a method to prevent emergence of resistant mutants;
- To further clarify the mechanism of macrophage-activation;
- To analyze surface antigens on macrophages using monoclonal antibodies and differentiate subpopulations of macrophages by such surface markers;
- To clarify the mechanism of antigen-presentation of macrophages to T-lymphocytes;
- To analyze the dynamics and roles of respective subsets of T-lymphocytes in order to better understand immunity and allergy in tuberculosis; cloned T-lymphocyte cell lines will be specifically useful in these studies;
- To study the dynamics of each subset of T-lymphocytes in human tuberculosis and atypical mycobacteriosis;
- To study host immunological factors to help determine the risk of developing tuberculosis or atypical mycobacteriosis;
- To clarify those factors which influence delayed-type hypersensitive reactions, particularly the contributions of suppressor T-lymphocytes and/or suppressor macrophages;
- To purify and characterize the chemical properties of lymphokines. Large quantities of pure lymphokine will be prepared by the method of cell-fusion or by cell-technology. *In vivo* dynamics and the role of each component of the lymphokine will be evaluated;
- To develop practical immunostimulants for tumor immunotherapy derived from tubercle bacilli; and
- To continue immunogenetic studies on native resistance and acquired immunity against tuberculosis infections.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
GRANTS	
T. Tokunaga Dep. of Cellular Immunology, National Inst. of Health	Studies on surface antigen on macrophages, on markers of activation of macrophages and on anti-tumor activity of DNA of tubercle bacilli
L. Toida Research Inst. of Tuberculosis, Japan Anti-Tuberculosis Association	Studies on the mechanism of resistance of mycobacteria against anti-tuberculous drugs Studies on glycolipids of tubercle bacilli
K. Saito Dep. of Microbiology, Keio Univ.	Studies on the mechanism of immunity in tuberculosis with cloned T-lymphocyte cell lines

Investigator and Institution	*Title*
K. Nomoto Dep. of Immunology, Medical Inst. of Bioregulation, Kyushu Univ.	Studies on the cellular immune mechanism in tuberculosis
I. Azuma Inst. of Immunological Sciences, Hokkaido Univ.	Studies on adjuvant-active substances of tubercle bacilli and on the anti-tumor activity of MDP-analogues
K. Yamamoto Inst. of Immunological Sciences, Hokkaido Univ.	Studies on the suppressive mechanism of delayed-type hypersensitivity by large amounts of antigen
K. Morikawa Inst. of Immunological Sciences, Hokkaido Univ.	Morphological and immunological studies on granuloma formation
K. Fukushi Dep. of Bacteriology, Hirosaki Univ.	Studies on the first structures of mycobacteria Studies on anti-tumor activities of cord factor
T. Hashimoto Inst. of Basic Medical Sciences, Univ. of Tsukuba	Studies on the purification of lymphokines and its properties Studies on the mechanism of macrophage-activation
K. Kanai National Inst. of Health	Studies on the pathogenesis of tuberculosis at the cellular and subcellular levels
S. Oshima Chest Disease Research Inst., Kyoto Univ.	Studies on the roles of macrophages in immunity in tuberculosis
A. Tanaka Dep. of Biochemistry, Shimane Medical Univ.	Studies on the mechanism of macrophage-activation by MDP
S. Kotani Dep. of Bacteriology, Dental School, Osaka Univ.	Studies on the adjuvant-active substances in bacterial cell wall
Y. Yamamura National Sanatorium Toneyama Hosp.	Studies on the mechanism in cavity-formation
K. Ueda Dep. of Veterinary Medicine, The Inst. of Public Health	Immunological studies on granuloma formation with nude mice
T. Yamaga Research Inst. for Microbial Diseases, Osaka Univ.	Studies on the mechanism of resistance of mycobacteria against anti-tuberculous drugs Studies on the genetics of mycobacteria
T. Tsuyuguchi Osaka Prefectural Habikino Hosp.	Studies on the immunity in tuberculosis in human, especially on the dynamics of T-lymphocytes in human tuberculosis
S. Morikawa Dep. of Pathology, Shimane Medical Univ.	Studies on the suppressive mechanism in delayed-type hypersensitivity
S. Fujimoto Dep. of Immunology, Koch Medical School	Studies on anti-tumor activity of mycobacterial constituents
R.M. Nakamura Dep. of Cellular Immunology, National Inst. of Health	Immunogenetical studies on the immunity and delayed-type hypersensitivity
I. Yano Dep. of Bacteriology, Niigata Univ.	Studies on mycolic acids of mycobacteria

Investigator and Institution	*Title*
Y. Mizuguchi Dep. of Microbiology, Univ. of Occupation and Environmental Health	Studies on the genetics of mycobacteria
S. Takatsu Dep. of Biology, Inst. for Medical Immunology, Kyushu Univ. Medical School	Studies on PPD-specific T-lymphocytes with cloned cell lines
S. Nagai Toneyama Inst. for Tuberculosis Research, School of Medicine, Osaka City Univ.	Studies on tuberculin antigen
K. Tabata Research Inst. of Tuberculosis, Japan Anti-Tuberculosis Association	Studies on the mechanism of hepatotoxicity of isoniazid
T. Kataoka Dep. of Cellular Immunology, National Inst. of Health	Studies on the mechanism of macrophage-activation and on the surface markers on macrophages

BIBLIOGRAPHY

Azuma, I., and Y. Yamamura. Antitumor activity of synthetic muramyl peptide derivatives. *Progress in Immunology V: Fifth International Congress on Immunology* (ed. by Y. Yamamura & T. Tada). Academic Press, Tokyo 1379–1382, 1982.

Azuma, I., K. Kamisango, I. Saiki, Y. Tanio, S. Kobayashi, and Y. Yamamura. Adjuvant activity of N-acetyl-muramyldipeptides for the induction of delayed-type hypersensitivity to azobenzenearsonate-N-acetyl-L-tyrosine in guinea pigs. *Infect. Immun.* *29*:1193–1196, 1980.

Fujimoto, S. Effect of BCG–CWS on the induction of immune resistance in low responder mouse against syngeneic tumor. *Proc. 16th Joint Conference on Tuberc.* U.S.-Japan Cooperative Med. Sci. Prog. (Supplement), 1981.

Fujimoto, S., K. Kitsukawa, T. Toko, and M. Shimizu. Effect of BCG–CWS on the induction of anti-tumor resistance in tumor-bearing hosts of either high or low responder mice. *Proc. 19th Joint Res. Conference on Tuberc.* U.S.-Japan Cooperative Med. Sci. Prog. (Supplement), 1984.

Fujiwara, H., Y. Okuda, T. Fukukawa, and I. Tsuyuguchi. *In vitro* tuberculin reactivity of lymphocytes from patients with tuberculous pleurisy. *Infect. Immun.* *35*:402–409, 1982.

Fukushi, K., H. Asano, and J. Sasaki. Antitumor activity of deacylated *Salmonella* endotoxin combined with trehalose dimycolate. *In: Immunomodulation by Microbial Products and Related Synthetic Compounds.* Y. Yamamura (ed.), Excerpta Medica, pp. 395–398, 1982.

Fukushi, K., H. Asano, J. Sasaki. Tumor regression induced by endotoxin combined with trehalose dimycolate. *In: Bacterial Endotoxin*, J.Y. Homma et al. (ed.), Verlag Chemie, pp. 251–260, 1984.

Goto, Y., R.M. Nakamura, H. Takahashi, and T. Tokunaga. Genetic control of resistance to *Mycobacterium intracellulare* infection in mice. *Infect. Immun.* *46*:135–140, 1984.

Hamaoka, T., K. Takatsu, K. Okuno, and T. Tsuchida. Functional characterization of the killer-helper factor responsible for the induction of cytotoxic T lymphocytes from thymocytes, and evidence for the nature of this factor as distinct from T cell-replacing factor (TRF) in regards to B cell triggering. *J. Immunol.* *127*:659–665, 1981.

Harada, K., S. Kotani, H. Takada, M. Tsujimoto, Y. Hirachi, S. Kusumoto, T. Shiba, S. Kawata, K. Yokogawa, H. Nishimura, T. Kitaura, and T. Nakajima. Liberation of serotonin from rabbit blood platelets by bacterial cell walls and related compounds. *Infect. Immun.* *37*:1181–1190, 1982.

Harboe, M., and S. Nagai. MPB70, a unique antigen of *Mycobacterium bovis* BCG. *Amer. Rev. Resp. Dis.* *129*:444–452, 1984.

Higuchi, S., M. Suga, A.M. Dannenberg, Jr., L.F. Affronti, I. Azuma, T.M. Daniel, and J.P. Petrali. Persistance of protein, carbohydrate and wax components of tubercle bacilli in BCG lesions. *Amer. Rev. Resp. Dis.* *123*:397–401, 1981.

Hiramine, C., K. Hojo, and I. Yano. Development of cell-mediated hypersensitivity in guinea pigs following infection with *Nocardia asteroides. Int. Archs Allergy Appl. Immun.* 65:220–234, 1981.

Homma, Y., K. Onozkai, Y. Fukutomi, and T. Hashimoto. The mechanism of cell surface changes of guinea pig macrophages activated with purified migration inhibitory factor/macrophage activation factor. *Cell. Immunol.* 72:231–238, 1982.

Homma, Y., K. Onozkai, T. Hashimoto, K. Miura, S. Nagao and A. Tanaka. Different effect of L-fucose binding lectin on macrophage migration inhibition caused by guinea pig migration inhibitory factor and synthetic muramyl dipeptide. *Int. Archs. Allergy appl. Immun.* 65:27–33, 1981.

Homma, Y., K. Onozkai, T. Hashimoto, Y. Nagai, and T. Takenawa. Differential activation of phospholipids metabolism of formylpeptide and ionophore A23187 in guinea pig peritoneal macrophages. *J. Immunol.* *129*:1619–1626, 1982.

Imai, K., M. Tomioka, S. Nagao, K. Kushima, A. Tanaka. Biochemical evidence for activation of guinea pig macrophages by muramyl dipeptide. *Biomedical Research* *1*:300–307, 1980.

Izumi, T., N. Fujimura, and S. Oshima. T-lymphocyte alveolitis and B-lymphocyte alveolitis: A new classification of interstitial pneumonitis based on broncho-alveolar lavage findings. *Asian Pacific J. Allerg. Immunol.* *1*:131–134, 1983.

Kamisango, K., I. Saiki, Y. Tanio, S. Kobayashi, T. Fukuda, I. Sekikawa, I. Azuma, and Y. Yamamura. Chemical synthesis and adjuvant activity of N-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) analogs. *Chem. Pharm. Bull.* 29:1644–1654, 1981.

Kanai, K., E. Kondo, and T. Yasuda. A plectron microscopy study of intra-cellular mycobacteria in experimental mouse tuberculosis. *Tubercle* *62*:187–195, 1981.

Kanai, K. and E. Kondo. Phospholipase-A_2-induced antimycobacterial activity in the membrane fraction from peritoneal exudate cells of guinea pigs. *Jap. J. Med. Sci. Biol.* *33*:87–101, 1980.

Kasai, M., T. Takashi, T. Takahashi, and T. Tokunaga. A new differentiation antigen shared with fetal thymocytes and leukemia cells in the mouse. *J. Exp. Med.* *159*:971–980, 1984.

Kato, K., T. Kataoka, T. Ohnuki, and T. Tokunaga. Establishment of macrophage-like cell lines of the guinea pig. *Microbiol. Immunol.* 25:1209–1214, 1981.

Kato, K., K. Yamamoto, T. Kimura, I. Azuma, and P.W. Askenase. Suppression of BCG cell wall-induced delayed-type hypersensitivity by pretreatment with killed BCG: Induction of nonspecific suppressor T cells by the adjuvant portion (MDP) and of specific suppressor T cells by the antigen portion (TAP). *J. Immunol.* *132*:2790–2795, 1984.

Kato, K., K. Yamamoto, H. Okuyama, and T. Kimura. Microbicidal activity and morphological characteristic of lung macrophages in *Mybobacterium bovis* BCG cell wall-induced lung granuloma in mice. *Infect. Immun.* *45*:325–331, 1984.

Kato, K., K. Yamamoto, M. Kakinuma, C. Ishihara, and I. Azuma. Suppression of BCG cell wall induced delayed-type hypersensitivity by BCG pre-treatment. I. Induction of adherent suppressor cells by live BCG injection and their characterization. *Immunology* *42*:259–266, 1981.

Kataoka, T., T. Komiyama, T. Ohnuki, M. Miyamoto, and T. Tokunaga. Natural killer (NK) cell activities of several inbred strains of guinea pig and their susceptibility to rabbit antiserum directed to guinea pig brain tissue. *Jpn. J. Med. Sci. Bio.* *35*:97–104, 1982.

Kataoka, T. and T. Tokunaga. Absence of detectable cytotoxic lymphocytes in the spleens from guinea pigs immunized with allogeneic cells. *Microbiol. Immunol.* *27*:619–629, 1983.

Kobayashi, S., T. Fukuda, H. Yukimasa, M. Fujino, I. Azuma, and Y. Yamamura. Synthesis, and the adjuvant and tumor-suppressive activities of quinonyl muramyl dipeptides. *Bull. Chem. Soc. Jpn.* *57*:3182–3196, 1984.

Kondo, E., T. Yasuda, and K. Kanai. Electron microscopic demonstration of close contact between intracellular mycobacteria and the phagosomal membrane. *Jap. J. Med. Sci. Biol.* *35*:197–201, 1982.

Kotani, S. *et al.* Lipophilic muramyl peptides and synthetic lipid A analogs as immunomodulators, *in* "Progress in Immunology V" (edited by Y. Yamamura, and T. Tada), 1359–1377, Academic Press Japan, Tokyo, 1983.

Kotani, S., I. Azuma, H. Takada, M. Tsujimoto, and Y. Yamamura. Muramyl dipeptides: Prospect for cancer treatments and immunostimulation *in* "Biological Response Modifiers in Human Oncology and Immunology" (edited by T. Klein, S. Specter, H. Friedman, A. Szentivanyi), 117–158, Plenum Press, New York, 1983.

Kotani, S., H. Takada, M. Tsujimoto, T. Kubo, T. Ogawa, I. Azuma, H. Ogawa, K. Matsumoto, W.A. Siddiqui, A. Tanaka, S. Nagao, O. Kohashi, S. Kanoh, T. Shiba, and S. Kusumoto. Nonspecific and antigen-specific stimulation of host defense mechanisms by lipophilic derivatives of muramyl dipeptides *in* "Bacteria and Cancer" (edited by J. Jeljaszewicz, G. Pulverer, and W. Roskowski), 67–107, Academic Press, London, 1982.

Kyuwa, S., K. Fujiwara, and K. Ueda. Suppression of BCG granuloma formation in mice by sensitized aplenic lymphocytes. *Japan J. Exp. Med.* *53*:69–72, 1983.

Matsumoto, T., S. Miake, M. Mitsuyama, K. Takeya, and K. Nomoto. Enhanced resistance to Listeria monocytogenes due to non-specifically activated macrophages in aged mice. *J. Clin. Lab. Immunol.* *8*:51–54, 1982.

Mitsuyama, M., K. Nomoto, and K. Takeya. Direct correlation between delayed footpad reaction and resistance to local bacterial infection. *Infec. Immun.* *36*:72–79, 1982.

Mitsuyama, M., K. Nomoto, H. Akeda, and K. Takeya. Enhanced elimination of Listeria monocytogenes at the site of delayed footpad reaction. *Infect. Immun.* *30*:1–4, 1980.

Miura, K., S. Nagai, M. Kinomot, S. Naga, and T. Tokunaga. Comparative studies with various substrains of *Mycobacterium bovis* BCG on the production of an antigeneic protein, MPB70. *Infect. Immun.* *39*:540–545, 1983.

Miyata, M., M. Mitsuyama, N. Ogata, K. Nomoto, and K. Takeya. Two steps in the generation of acquired cellular resistance against Listeria monocytogenes: accumulation and activation of macrophages. *Immunology* *47*:247–253, 1982.

Miyata, M., M. Mitsuyama, N. Ogata, and K. Nomoto. Protective mechanisms against infection by *Listeria monocytogenes*: Accumulation and activation of macrophages. *J. Clin Lab. Immunol.* *13*:111–115, 1984.

Mizugucui, Y., T. Udou, and T. Yamada. Mechanism of antibiotic resistance in *Mycobacterium intracellulare*. *Microbiol. and Immunol.* *27*:425–431, 1983.

Mizushima, A., T. Baba, T. Ochiya, K. Yamaguchi, K. Onozaki, H. Yaoita, and K. Ueno. Regulatory mechanisms of cutaneous delayed-type hypersensitivity. I. Suppression of cutaneous delayed-type hypersensitivity by migration inhibitory factor. *Cell. Immunol.* *81*:126–133, 1983.

Mizuguchi, Y., M. Fukunaga, and H. Taniguchi. Plasmid deoxyribonucleic acid and translucent to opaque variation in *Mycobacterium intracellulare* 103. *J. Bacteriology* *146*:656–659, 1981.

Morikawa, S. CY-effect. *Progress in Medicine* *3*:1613–1623, 1983.

Nagai, S., J. Matsumoto, and T. Nagasuga. Specific skin-reactive protein from culture filtrate of *Mycobacterium bovis* BCG. *Infect. Immun.* *31*:1152–1160, 1981.

Nagai, S., T. Nagasuga, and J. Matsumoto. Tuberculin peptide from culture filtrate of *Mycobacterium tuberculosis*. *Amer. Rev. Resp. Dis.* *121*:551–557, 1980.

Nagao, S., and A. Tanaka. Inhibition of macrophage DNA synthesis by immunomodulators. I. Suppression of [^{3}H] thymidine incorporation into macrophages by MDP and LPS. *Microbiol. Immunol.* *27*:377–387, 1983.

Nakamura, R.M., H. Tanaka, and T. Tokunaga. Strain difference in delayed-type hypersensitivity to BCG in mice: role of splenic adherent cells in the primary immune response. *Immunology* *47*:729–731, p. 1982.

Nakamura, R.M., and T. Tokunaga. I-J positive macrophages in induction of suppressor T cells against delayed-type hypersensitivity to *Mycobacterium bovis* BCG. *Microbiology* *1984*:363–365.

Nakamura, R.M., H. Tanaka, and T. Tokunaga. *In vitro* induction of suppressor T-cells in delayed-type hypersensitivity to BCG and an essential role of I-J positive accessory cells. *Immunol. Lett. 4*:295–299, 1982.

Nakamura, R.M., and T. Tokunaga. Induction of suppressor T cells in delayed-type hypersensitivity to *Mycobacterium bovis* BCG in low-responder mice. *Infect. Immun. 28*:331–335, 1980.

Nomoto, K., Y. Shimamoto, K. Taniguchi, C. Kubo, H. Kawauchi, M. Mitsuyama, and K. Takeya. Development of immunity against Listeria monocytogenes in athymic nude versus neonatally thymectomized mice. *Cell. Immuno. 75*:134–143, 1983.

Ochiya, T., T. Baba, A. Mizushima, K. Onozaki, and H. Yaoita. Induction of macrophage disappearance reaction by immunoadsorbed MIF. *Cell. Immunol. 71*:346–352, 1982.

Ochiya, T., T. Baba, K. Onozaki, H. Yaoita, K. Ueno, and T. Hashimoto. Regulatory mechanisms of cutaneous delayed-type hypersensitivity 2. Suppression of cutaneous delayed-type hypersensitivity by macrophage disappearance reaction. *Cell Immunol. 81*:134–143, 1983.

Ogawa, T., S. Kotani, K. Fukuda, Y. Tsukamoto, M. Mori, S. Kusumoto, and T. Shiba. Stimulation of migration of human monocytes by bacterial cell walls and muramyl peptides. *Infect. Immun. 38*:817–820, 1982.

Okuyama, H., S. Kobayashi, M. Fujita, R. Yasumizu, and K. Morikawa. Tissue localization of complement component 3 receptor-bearing cells in lymphoid tissue after injection with the complete Freund Adjuvant. *Infect. & Immun. 38*:724–730, 1982.

Onozaki, K., S. Akagawa, S. Haga, K. Miura, and T. Hashimoto. Role of lymphokines in regulation of macrophage differentiation. *Cell. Immunol. 76*:129–136, 1983.

Onozaki, K., S. Haga, M. Ichikawa, Y. Homma, K. Miura, and T. Hashimoto. Production of an antibody against guinea pig MIF. III. Biological activity of MIT recovered from immunoadsorbent column chromatography. *Cell. Immunol. 61*:165–175, 1981.

Onozaki, K., S. Haga, K. Miura, Y. Homma, and T. Hashimoto. Production of an antibody against guinea pig MIF. II. Analysis of the antibody-reacting material using radiolabelled lymphokines. *Cell. Immunol. 55*:465–475, 1980.

Onozaki, K., K. Suzuki, Y. Fukutomi, K. Yagami and T. Hashimoto. Activation of guinea pig macrophages with a Streptococcal preparation OK-432 and its augmentation by stimulated spleen cell products. *J. Reticuloendothel. Soc. 32*:433–442, 1982.

Onozaki, K., Y. Homma, K. Miura, and T. Hashimoto. Scanning electron microscopic study of the cell surface changes of lymphokine-treated guinea pig macrophages. *J. Reticuloendothel Soc. 30*:471–481, 1981.

Onozaki, K., T. Takenawa, Y. Homma, and T. Hashimoto. The mechanism of macrophage activation induced by CA^{2+} ionophore. *Cell. Immunol. 75*:242–254, 1983.

Saiki, I., M. Uemiya, T. Kusama, Y. Yamamura, and I. Azuma. Adjuvant activity of nocardomycoloyl derivatives of N-acetylmuramyl-dipeptides in mice and guinea pigs. *Microbiol. Immunol. 24*:265–269, 1980.

Saito, K. and H. Narimatsu. Adverse effect of the transstimulation on a long-term culture of PPD-specific T cells. *Proc. of 16th Joint Conference on Tuberc., U.S.-Japan Cooperative Med. Sci. Prog.*:394–408, 1981.

Saito, K., T. Tadakuma, and H. Narimatsu. Establishment of PPD-specific cloned murine T cell lines, and characteristics of the cloned T cells. *Proc. of 17th Joint Meeting of Tuberc. Panel, U.S.-Japan Cooperative Med. Sci. Prog.*:236–245, 1982.

Saito, K., T. Tadakuma, and H. Narimatsu. PPD-specific murine T-cell clone: Properties of the cloned T cells in the elicitation of delayed-type hypersensitivity and the protective immunity to intracellular bacterial infection. *Proc. of 18th Joint Conference on Tuberc., U.S.-Japan Cooperative Med. Sci. Prog.*:208–221, 1983.

Sano, Y., G. Yamada, K. Dobashi, T. Mizuochi, T. Hamaoka, and K. Takatsu. Establishment of three PPD-reactive T cell clones with distinct functions in B cell activation. *J. Immunol. 133*:629–635, 1984.

Shiratsuchi, H. and I. Tsuyuguchi. Tuberculin purified protein derivative-reactive T cells in cord blood lymphocytes. *Infect. Immun. 33*:651–657, 1981.

Shiratsuchi, H. and I. Tsuyuguchi. Analysis of T cell subsets by monoclonal antibodies in patients with tuberculosis after *in vitro* stimulation with purified protein derivative of tuberculin. *Clin. Exp. Immunol. 57*:271–278, 1984.

Tabata, K., S. Yamamoto, I. Toida. Isoniazid metabolism related with hepatotoxicity. *Proc. of 19th Joint Res. Conference on Tuberc., U.S.-Japan Cooperative Med. Sci. Prog*:189–210, 1984.

Takade, A., K. Takeya, H. Taniguchi, and Y. Mizuguchi. Electron microscopic observation of cell division in *Mycobacterium vaccae* VI. *J. General Microbiol. 129*:2315–2320, 1983.

Takatsu, K., A. Tominaga, T. Hamaoka. Antigen-induced T cell-replacing factor (TRF). I. Functional characterization of a TRF-producing helper T cell subset and genetic studies on TRF production. *J. Immunol. 124*:2414–2422, 1980.

Takatsu, K., T. Homaoka, A. Tominaga, and Y. Kanamasa. Augmented induction of tumor-specific resistance by priming with Mycobacterium tuberculosis (Tbc) and subsequent immunization with PPD-coupled syngeneic tumor cells. *J. Immunol. 125*:2367–2373, 1980.

Tanaka, A., K. Emori, S. Nagao, K. Kushima, O. Kohashi, M. Saitoh, and T. Kataoka. Epithelioid granuloma formation requiring no T-cell function. *Am. J. Pathol. 106*:165–170, 1982.

Tanaka, A., S. Nagao, K. Imai, R. Mori. Macrophage activation by muramyl dipeptide as measured by macrophage spreading and attachment. *Microbiol. Immunol. 24*:547–557, 1980.

Taniyama, T., and T. Tokunaga. Monoclonal antibodies directed against mouse macrophages in different stages of activation for tumor cytotoxicity. *J. Immunol. 131*:1032–1037, 1983.

Toida, I., A. Misaki, and N. Kataoka. A glycolipid of Mycobacterium tuberculosis, strain H_{37}Rv. *Proc. of 16th Joint Conference on Tuberc., U.S.-Japan Cooperative Med. Sci. Pro.* (Supplement), 1981.

Toida, I., and S. Yamamoto. Inactivation of isoniazid by mycobacteria. *Proc. of 18th Joint Conference on Tuberc., U.S.-Japan Cooperative Med. Sci. Prog.*, 349-367, 1983.

Tokunaga, T., H. Yamamoto, S. Shimada, H. Abe, T. Fukuda, Y. Fujisawa, Y. Furutani, O. Yano, T. Kataoka, T. Sudo, N. Makiguchi, and T. Suganuma. Antitumor activity of deoxyribonucleic acid fraction from *Mycobacterium bovis* BCG. I. Isolation, physicochemical characterization and antitumor activity. *J. Natl. Cancer Inst.*, *72*:955–962, 1984.

Tokunaga, T. and K.S. Akagawa. A cell surface marker expressed on cytotoxic peritoneal macrophages and normal lung macrophages *in* "Macrophages and Natural Killer Cells: Regulation and Function" (S.J. Normann, E. Sorkin, eds.), 429–434, Plenum Publ. Co., 1982.

Tominaga, A., K. Takatsu, T. Hamaoka. Antigen-induced T cell-replacing factor (TRF). II. X-linked gene control for the expression of TRF-acceptor site(s) on B lymphocytes and preparation of specific antiserum to that acceptor. *J. Immunol. 124*:2423–2429, 1980.

Tomiyasu, I., and I. Yano. Isonicotinic acid hydrazide induced changes and inhibition in mycolic acid synthesis in *Nocardia* and related taxa. *Arch. Microbiol. 137*:316–323, 1984.

Tomiyasu, I., and I. Yano. Separation and analysis of novel polyunsaturated mycolic acids from a psychrophilic, acid-fast bacterium, *Gordona aurantiaca*. *Eur. J. Biochem 139*:173–180, 1984.

Tomiyasu, I., S. Toriyama, I. Yano, and M. Masui. Changes in molecular species composition of nocardomycolic acids in *Nocardia rubra* by the growth temperature. *Chem. Phys. Lipids 28*:41–54, 1981.

Toriyama, S., S. Imaizumi, I. Tomiyasu, M. Masui, and I. Yano. Incorporation of ^{18}O into long-chain, secondary alcohols derester mycolic acids in *Mycobacterium phlei*. *Bioch. Biophy. Acta 712*:427–429, 1982.

Toriyama, S., I. Yano, M. Masui, E. Kusunose, M. Kusunose, and N. Akimori. Regulation of cell wall mycolic acid biosynthesis in acid-fast bacteria. I. Temperature-induced changes in mycolic acid molecular species and related compounds in *Mycobacterium phlei*. *J. Biochem. 88*:211–221, 1980.

Tsuyuguchi, I., H. Shiratsuchi, H. Fujiwara. Increase in rosette-forming T cells with autologous human erythrocytes in lymphocytes of patients with tuberculosis by *in vitro* stimulation with purified protein derivative. *Int. Archs Allergy Appl Immun. 67*:161–168, 1982.

Tsuyuguchi, I., H. Shiratsuchi, H. Fujiwara, O. Teraoka. Nonspecific recruitment of lymphocytes in purified protein derivative-induced lymphocyte proliferative response of patients with tuberculosis. *Infect. Immun. 37*:702–709, 1982.

Tsuyuguchi, I., H. Shiratsuchi, O. Teraoka, T. Hirano. Increase in T cells bearing IgG Fc receptors in peripheral blood of patients with tuberculosis by *in vitro* stimulation with purified protein derivative. *Amer. Rev. Resp. Dis. 121*:951–957, 1980.

Udou, T., M. Ogawa, and Y. Mizuguchi. Spheroplast formation of *Mycobacterium smegmatis* and morphological aspects of their reversion to the bacillary form. *J. Bact.*, *151*:1035–1039, 1982.

Udou, T., M. Ogawa, and Y. Mizuguchi. An improved method for the preparation of mycobacterial spheroplasts and the mechanism involved in the reversion to bacillary form: electron microspic and physiological study. *Canad. J. Microbiol. 29*: 60–68, 1983.

Ueda, K., S. Yamazaki, S. Yamamoto, and S. Someya. Spleen cell transfer induces T cell-dependent granulomas in tuberculous nude mice. *Journal of the Reticuloendothelial Society 31*:469–478, 1982.

Ueda, K., S. Yamazaki, S., and S. Yamamoto. H-2 I region restriction phenomenon in T cell-dependent granoloma formation to Mycobacterium bovis BCG. *Journal of the Reticulendothelial Society 34*:475–484, 1983.

Yagawa, K., M. Kaku, Y. Ichinose, S. Nagao, A. Tanaka, A. Tomoda. Biphasic effects of muramyl dipeptide or lipopolysaccharide on superoxide anion-generating activities of macrophage. *Infect. Immun. 45*:82–86, 1984

Yamada, A., K. Himeno, H. Miyata, Y. Kumazawa, and K. Nomoto. Antigen-specific augmentation factor involved in murine delayed-type footpad reaction. I. Nature of augmentation factor. *Cell. Immunol. 88*:184,192, 1984.

Yamada, A., K. Himeno, Y. Kumazawa, and K. Nomoto. Antigen-specific augmentation of delayed-type hypersensitivity by a humoral factor in the culture supernetant of immune spleen cells. *Cell. Immunol 84*:206–209, 1984.

Yamada, T., T. Yamanouchi, Y. Ono, and A. Nagata. Antibacterial activity of palmitoyltuberactinamine N and di-β-lysylcapreomycin IIA. *J. Antibiotics*, *36*:1729–1734, 1983.

Yamada, T., T. Yamanouchi, A. Nagata, and Y. Ono. The progress in the study on mode of action of antituberculous antibiotics in drug-sensitive and -resistant mycobacterial organism. 5th International Symposium of Plasmids (Czechoslovakia), Abstract form, 1983.

Yamada, T., T. Yamanouchi, A. Nagata, Y. Suzuki, and Y. Mizuguchi. The mode of action of antituberculous drug. Czechoslovakia Publ.

Yamada, T., T. Teshima, and T. Shiba. tRNA binding to programmed ribosomes increases the ribomal affinity for tuberactinomycin O. *FEBS LETTERS*, in press.

Yamada, T., T. Teshima, T. Shiba, and K.H. Nierhaus. The translocation inhibitor tuberactinomycin binds to nucleic acids and the *in vitro* assembly of 50S subunits. *Nucleic Acid. Res.* *8*:5767–5777, 1980.

Yamada, T., T. Yeshima, and T. Shiba. Activity of di-β-Lysyl-capreomycin IIA and palmitoyl-tuberactinamine N against drug-resistant mutants with altered ribosomes. *Antimicrob. Agents Chemother.* *20*:824–836, 1981.

Yamada, T., K.H. Nierhaus, T. Shiba, Y. Mizuguchi, and T. Yamanouchi. Mode of action of viomycin. Drug resistance in Bacteria (Japan Scientific Societies Press, Tokyo, Thieme-stratton Inc., New York) 1981.

Yamada, T., K.H. Nierhaus, T. Teshima, T. Shiba, Y. Mizuguchi, and T. Yamanouchi. Mode of action of viomycin and biochemical mechanism of viomycin resistance. The 3rd Tokyo Symposium on Microbial Drug-resistance, Abstract form, 1981.

Yamada, T. Characterization of an Sl-like protein in *Mycobacterium smegmatis* ribosomes. *FEBS LETTER* *142*:267-270, 1982.

Yamamoto, K., K. Kato, M. Kakinuma, H. Okuyama, and I. Azuma. Further study on relationship of anti-tuberculous protection to lung granulomata produced by intravenous injections of synthetic 6-0-mycoloyl-N-acetylmuramyl-L-alanyl-D-isoglutamine with or without specific antigens. *Immunology* *46*:473–479, 1982.

Yamamoto, K., K. Kato, M. Kakinuma, and W. Brehmer. Cellular regulation of lung granuloma formation and delayed-type hypersensitivity induced with BCG cell walls. Further evidence of involvement of cellular immunity in lung granuloma formation. *Zbl. Bakt. Hyg., I. Akt. Orig., A 251*:357–368, 1982.

Yamanouchi, K., H. Ohta, T. Kataoka, F. Kobune, Y. Yoshikawa, and T. Tokunaga. Suppression of delayed hypersensitivity by measles virus infection in guinea pigs. *Jpn. J. Med. Sci. Biol.* *34*:81–94, 1981.

Yamazaki, S., K. Ueda, and S. Someya. Non-immunological resistance of nude mice to infection with a virulent strain of Mycobacterium bovis *in* "Recent advances in germ-free research" (Sasaki, S., Ozawa, A., and Hashimoto, K. eds.), 419–423, Tokai University Press, Tokyo, 1981.

Yasumizu, R., K. Onoe, M. Ogasawara, K. Iwabuchi, Geng Li, and K. Morikawa. Contact sensitivity in fully allogeneic bone marrow chimera in mice. *Asian Pacif. J. Allerg. & Immunol.* *2*:61–66, 1984.

Yoshikai, Y., S. Miake, T. Matsumoto, K. Nomoto, and K. Takeya. Relationship between non-specific activity of macrophages and immune responses to Listeria monocytogenes. *Immunology*, *40*:295–301, 1980.

U.S. Tuberculosis Panel

BROAD GOALS

Tuberculosis ranks among the foremost of infectious diseases to afflict mankind. The World Health Organization estimates that as many as three million victims die of the disease annually. With currently available technology for immunization (principally with the Bacillus Calmette-Guerin vaccines), realistic prospects of eradicating tuberculosis are meager. Indeed, the dismal failure of a recently evaluated BCG vaccination field trial, sponsored by WHO in the Chingleput region of South India, elicited a concerned reappraisal of the immunization program. Consequently, a new ambitious and highly sophisticated program on the immunology of tuberculosis was recently launched. This reaffirms the original and ongoing commitment of the U.S.-Japan CMSP to clarify such mechanisms which complements other approaches relevant to elucidating the pathogenesis of tuberculosis. Throughout the tenure of the U.S.-Japan Tuberculosis Programs understanding and manipulation of these two phenomena, immunology and pathogenesis, have been the major objectives.

SHORT SUMMARY OF THE FIRST FIFTEEN YEARS

From inception of the program it was conceived that chemotherapy and clinical research of tuberculosis were receiving adequate attention. These categories were specifically excluded from the panel's committed interests in favor of pursuing studies of tuberculous immunity (including prevention) and pathogenesis. These were conceived as potential guides for favorably altering the host immune response.

For effective immunity the response of specifically committed lymphocytes to the stimulus of specific antigens results in the production and release of lymphokines. These include migration inhibiting factor (MIF) and macrophage activating factor (MAF), which are recognized by glycolipid receptors on the macrophage membrane surface. Similar substances are found in sera of BCG-immunized mice when challenged intravenously with tuberculin. Interaction of the macrophage membrane surface with lymphokines stimulates macrophages to an activated state so that the intracellular multiplication of *M. tuberculosis* (and non-specifically, of some other microorganisms) is suppressed. Earlier it was shown that these activated macrophages are cidal for certain tumor cells.

In vaccine research, subhuman primates were effectively protected against aerogenic tuberculosis challenge if vaccinated with viable BCG (optimally 10^5 viable units) by inhalation, or aerosolization of droplet nuclei containing a few virulent bacilli. In the active immunized state, protection is probably expressed through suppression of a hematogenous spread originating from the primary lesions. This is prominent in the seedling of the subapical regions of the lungs.

Other studies involve vaccine effectiveness comprising BCG cell walls wet with mineral oil and emulsified into Tween-saline. An impressive parallel was drawn between the protective behavior of this “vaccine” when injected intravenously in experimental animals and that induced by viable BCG. Extension of this concept to treatment of experimental animal tumors (which responded favorably to intratumor injection with viable BCG) led to the development of very effective antitumor vaccines with non-viable components. Such experimental studies and their possible significance were thoroughly discussed in an earlier report (1970–75). The therapeutic analysis of the various components showed that the attenuated activity of such vaccines which contained “cell wall skeleton,” instead of whole BCG cell walls could be reinforced to full activity by incorporating the toxic mycobacterial glycolipid—trehalose-6, 6^1-dimycolate, into the vaccine. This glycolipid is known as “cord factor” or “P3.” The three terms are synonymous. To study structure-activity relations and seek more economical substitutes for trehalose dimycolate in such applications, an extensive program of synthesis yielded useful lipid amide derivatives of “trehalose dicarboxylic acid.” These were considerably less toxic than cord factor but adequately substituted for this glycolipid in select antitumor vaccines. In other studies centering on mycobacterial trehalose derivatives, the complete structures of two sulfatides of *M. tuberculosis* were established. They were provocatively implicated as possible contributors to the intracellular survival of virulent tubercle bacilli; hence, they are involved in pathogenesis of tuberculosis. The sulfatides antagonize phagosome-lysosome fusion in macrophages—a phenomenon which mimics the behavior of the tubercle bacillus itself.

For over a decade the panel supported extensive efforts to produce antigens from *M. tuberculosis* that would serve as specific “tuberculins” applicable for epidemiologic and diagnostic studies. The most sophisticated techniques of fractionation and separation of cell extract and culture filtrate components, derived from cultivation of *Mycobacterium tuberculosis*, yielded only products which cross-reacted with antigens from a host of non-tuberculosis mycobacteria. When tested in experimental animals sensitized with these heterologous mycobacteria they showed considerable cross reactivity. Interpretation of these reactions is described later.

ADMINISTRATIVE DEVELOPMENTS IN THE CURRENT FIVE-YEAR PERIOD

Since the activities of the U.S.-Japan tuberculosis program cannot realistically embrace every conceivable aspect of the problem, sober assessments at the time of the program's inception dictated that the principal efforts targeted at curtailing the disease should focus on prevention rather than cure. In retrospect, with the possible exceptions of disease attributable to the “atypical” (nontuberculosis) mycobacteria, this goal was well chosen, in that it brought the supported research efforts into the mainstream of the prodigious advances in cellular immunology that evolved during the last decade. This is altogether appropriate: mycobacteria have been and continue to be an indispensable commodity for the immunologist. It was in accord with this undeniable status therefore, that as one facet of the effort, the goals of the Tuberculosis Panels were expanded to embrace studies on the roles that mycobacterial products might play in tumor immunology and tumor immunotherapy. The immediate practical benefits that accrued from such ventures cannot be denied. But clearly the pendulum reached such a high arc that other, equally compelling areas of research, perhaps more relevant to tuberculosis, suffered from dilution; and the program was in danger of being too grossly diverted and of being consumed by its own offspring.

The precarious status was recognized and it was relieved during the period covered by this report by two interventions: directly on one hand, by a reassessment and reformulation of the objectives of the Tuberculosis Panels; and indirectly on the other, by the timely and appropriate founding of an additional "panel" of the U.S.-Japan Cooperative Medical Science Program—the Immunology Board—an action under serious consideration at the time of the last review and which was approved and implemented during the current period.

As yet another administrative change the combined United States and Japanese Delegations recommended that the activities of the Tuberculosis and Leprosy Panels should in the future be much more closely coordinated, although not physically merged. This is a welcomed compromise, a laudable alternative to that of combining the two panels, where efforts as a single unit would become diluted and diffuse. Although the two panels have a number of common interests, there are also important differences. On the other hand, the glue which holds the mutual interests together is the fact that *M. leprae* is a member of the same genus (although there are dissenting opinions on this point); and recent studies have already revealed similarities in chemical compositions (elaboration by both *M. tuberculosis* and *M. leprae* of phthiocerol dimycocerosate, mycolic acids, mycoside-like glycolipids, etc.). These and other common interests will be shared, and will catalyze and promote the efforts of the panels in the future.

PROGRESS IN PRIORITY AREAS

Tuberculins and Other Antigens

There were numerous attempts to isolate monospecific antigens for *M. tuberculosis* using various physico-chemical procedures of high resolving potential. Affinity chromatographic procedures using hyperimmune immunoglobulins toward various species of mycobacteria substracted numerous components from *M. tuberculosis* sonicates; specificity increased only marginally. Chromatography with apparent immobilized specific antiserum resulted in fractions which were not monospecific. Rocket immunoelectrophoresis (IE) and two dimensional IE revealed that the degree of antigenic sharing for even distantly related species was considerable (e.g. about 30%). This permits better understanding of the cross sensitization induced by nontuberculosis species to tuberculin in various geographic areas.

In other studies antigen 5 (U.S.-Japan nomenclature) appeared to be a highly specific protein, apparently limited in distribution to *M. tuberculosis* and *M. bovis*. Immunoabsorbent affinity chromatography allowed isolation of Ag 5 in a high state of purity. It was extensively characterized. A field trial was conducted in 1,900 subjects in the United States and Argentina in which the specificity of antigen 5, as a tuberculin skin test antigen, was compared with tuberculin purified protein derivative (PPD). Results of this study showed that antigen 5, used in doses which were bioequivalent to intermediate strength PPD, is no more specific than PPD in populations with a high prevalence of nonspecific PPD reactivity.

Antigen 5 may be suitable for use in enzyme-linked immunosorbent assays (ELISA) of serum IgG antibody. Its use for serodiagnosis has been evaluated in 284 patients from Bolivia, Argentina, China, and Cleveland and 382 control subjects from the same areas. In two groups of patients geometric mean titers ranged from 1:64 to 1:183. In control groups geometric mean titers ranged from 1:4 to 1:31. X-ray data, where available, suggest that higher titers relate to the extent of pulmonary tuberculosis. ELISA studies also provided the opportunity to study sera from 21 patients with mycobacterial infections other than tuberculosis. Seven reacted with titers exceeding 1:80, which suggests a lack of species specificity. This parallels that observed in skin testing.

In persons infected with other mycobacteria there is an apparent paradox between distribution of antigen 5 to *M. tuberculosis* and *M. bovis* interactivity to skin test and ELISA systems. Monoclonal antibody studies are being used to resolve this discrepancy.

Most antigens of mycobacteria are multideterminant and may contain, in addition to specific epitopes, some which are shared by antigenic molecules of other species. Early monoclonal antibody studies have provided supportive evidence for this interpretation. They indicate that antigen 5 contains at least one epitope shared with other mycobacterial antigens.

In contrast to the intractability of the protein antigens to yield specific products, serologically active lipids of various nontuberculosis mycobacteria have unique and very useful specificities. The *M. avium, intracellulare, scrofulaceum* (MAIS) complex is distinguished by elaborating serologically specific lipid antigens; complete structures were obtained in several cases. All members have an almost invariant peptido-glycolipid core essentially identical with the simple, well known C-mycosides: a fatty acyl group amide-linked to a rhamnose-terminated small peptide; viz. Acyl-D-Phe-D-aThr-D-Ala-L-alol-O-methyl rhamnose. Appended to the aThr is a glycosidically linked acetylated deoxytalose to which small (2 or 3 sugars) oligosaccharides are attached. These provide the serological specificity. When rabbits are injected with killed bacilli of a given serovar—(developed by the late Werner B. Schaefer)—lipids are presented in a configuration adequate to elicit a very good antibody response. Antisera obtained in this way have been exploited for years in seroagglutination to identify the individual serovars. Specificity is conferred by the small oligosaccharides on the allo-threonine.

Novel glycolipid antigens, distinct from the C-mycoside type, have been isolated and characterized from other "atypical" mycobacteria: *M. kansasii, M. szulgai, M. xenopi, M. gordonae*, etc. In *M. kansasii* a core tetrasaccharide terminating in trehalose is distally substituted with a single rhamnose and then with increasing numbers of xylose molecules. Specific antigenicity is conferred when the oligosaccharide incorporates both an L-fucose and a novel N-acetylamino deoxy sugar as the terminal substituents. The structure of the amino deoxy sugar was determined and is named "N-acetyl kansosamine." Acyl groups on the tetra saccharide provide the lipid character. There is no evidence that these antigenic lipids elicit an antibody response in an infected host. However, their serological specificity represents a likely powerful tool useful for unequivocal taxonomic identification of a remarkable spectrum of mycobacteria.

The United States Tuberculosis Panel provided major financial support for an *International Conference on Atypical Mycobacteria*. The symposium held September 5–8, 1979, at the National Jewish Hospital and Research Center/National Asthma Center, Denver, Colorado, was dedicated to the memory of Dr. Werner B. Schaefer in recognition of his outstanding contributions to this field. The entire proceedings were published in *Reviews of Infectious Disease 3* (5) 813–1114 (1981), Paul T. Davidson, editor.

Chemistry of Mycobacteria; Mechanisms in Pathogenesis

Trehalose-containing glycolipids, a hallmark of the CMN genera (Corynebacteria, Mycobacteria, Nocardia) received intense study. Representative lipids are cord factor (trehalose-6,6^{1}-dimycolate-"TDM"); trehalose-6--monomycolate ("TMM"); and the sulfatides (sulfolipids-"SL"), which are relatively restricted to the more virulent strains of *M. tuberculosis*. Biological activities such as adjuvanticity, granulomagenicity, murine toxicity—of TDM presumably depend upon the physical state in which the lipid is presented at an oil-aqueous interface or when adsorbed as a monolayer on hydrophobic beads. In these forms TDM is described as selectively adsorbing fibrinogen in a reactive "end-on" fashion, with the long axis of the protein perpendicular to the interface. This orientation promotes thrombin-mediated release of chemotactic fibrinopeptide B. It in turn attracts inflammatory cells and results in secretion of more thrombin-like material. The combination of inflammatory cells, resistance of the adsorbed fibrinogen residue to proteolysis or macrophage processing to granuloma formation and disposition to hemorrhage, all represent typical manifestations of cord factor toxicity.

Trehalose monomycolate has assumed considerable prominence. After discovery by the Dr. Masahiko Kato its chemical structure was achieved in the laboratory of an American collaborator. French colleagues of the late Dr. Zulema Reggiado recognized that her serologically active glycolipid (SAG) antigen A_1 from BCG is TMM. Natural TMM, synthetic TMM and synthetic lower molecular weight analogs (containing corynomycolic acid) function equally well in passive hemagglutination tests to detect antibodies to TMM. This glycolipid is ubiquitous in mycobacteria and is very strong evidence based on thin-layer chromatography and infrared spectrophotometry, that TMM or a very closely related substance is elaborated even by *M. leprae*. Other reports which claim presence of trehalose dimycolate in *M. leprae* lipids appear to be premature. *M. leprae* may be a curious exception in possibly not elaborating this important glycolipid. TMM, or a very closely related material, probably transfers newly synthesized mycolic acid to the growing cell wall. It is very satisfying to find this substance, apparently without exception, among the lipids of this genus.

Promising new pseudo cord factors and their biologic activities have been assessed. Synthetic forms of natural cord factor and TMM were developed. The ultimate assessment of stereochemical requirement for cord factor toxicity was achieved: dimycolates of mannose-mannoside and galactose-galactoside were synthesized and found to have remarkably attenuated mouse toxicity. *In vitro* tests show these lipids to attack mitochrondrial membranes as effectively as cord factor. They disrupt electron transport along the respiratory chain and abolish oxidative phosphorylation. Hence, these processes are probably not involved in the expression of cord factor toxicity.

Intriguing new pathogenic mechanisms for *M. tuberculosis* have been proposed. Virulent organisms prevent phagosome-lysosome fusion in macrophages. Recently several electron microscopic studies provided intriguing evidence that the more virulent strains may also escape the confines of the phagosome, possibly by rupturing the membrane. Cells of older, and especially those from immunized, animals are notably resistant to this phagosomal disruption.

Mycolic acids and cell wall biosynthesis: mode of action of isoniazid

Recent studies resolved various remaining questions about their structures, based on total synthesis of several mycolic acids. Other studies with *M. tuberculosis* H37Ra clarified the biosynthetic pathways for elaboration and utilization of these remarkable high molecular weight (about 90 carbon atoms) substances. The chemical structures of the dicylopropyl (α)mycolic acids and those of important C_{32} to C_{56} and C_{24} to C_{32} precursors were established. The latter, as unsaturated fatty acids, are stringently required for the sequential biosynthetic reactions; it was found that Isoniazid (INH) inhibited their synthesis. Conceivably this represents the site of drug actions. Since mycolic acids comprise an important component of the mycobacterial cell wall, antagonism of their synthesis is a lethal event.

Immunology: human tuberculoimmunity; BCG vaccine

An intriguing method for assessing human tuberculoimmunity *in vitro* is under investigation. It depends on the concept that macrophages infected in culture with *M. tuberculosis* allow relatively unrestricted bacillary multiplication unless the phagocytic cells are appropriately "activated" by immune lymphokines. A donor's peripheral blood monocytes and lymphocytes are cultured separately for several days. The former develop into macrophages; the latter, if from an "immune" donor and incubated with bacillary antigen, produce immune lymphokines. The macrophages are infected with tubercle bacilli and divided into cultures incubated with the autologous lymphokines or with supernates as controls. Tuberculoimmunity is confirmed by restricted growth and degeneration of the acid fast bacilli in the "immune" supernate cultures as compared with the control samples.

An earlier comment referred to the recent failure of a very extensive WHO-sponsored field trial of BCG vaccination in South India. Studies in animals are testing various hypotheses to explain the results. Guinea pigs infected with tubercle bacilli, obtained from sputum of individuals in the Chingleput District, gave results which permitted exclusion of the following explanations: 1) that the vaccines were of poor quality; 2) that BCG doesn't protect against tubercle bacilli indigenous to the Chingleput region; and 3) the trail failure is not explained by the thesis that widespread universal infection with environmental (atypical) mycobacteria provided a protective effect nearly as strong as that of BCG. Despite a virtual saturation level of infection with environmental mycobacteria, the Chingleput District was selected for the trial because of its very high incidence of tuberculosis. Under evaluation is the hypothesis that exogenous infection or reinfection occurs by chance implantation of bacilli in a "privileged site:" establishment of a lesion in the apical—subapical lung region in the face of an otherwise effective cell-mediated immunity.

In other studies mice immunized by the airborne route with *Listeria monocytogenes* or *Mycobacterium bovis* BCG were more resistant to airborne challenge with the homologous organism than mice immunized by the intravenous (IV) or subcutaneous (SC) routes. All groups of mice were equally resistant to IV or SC challenge. Thus, airborne infection left an imprint on the lungs in contrast to other routes of infection. Lungs of animals immunized with BCG contained nodules of lymphoid cells that possibly confer subsequent resistance to homologous infection. This hypothesis was tested by transferring lymphocytes isolated from BCG-infected lungs to naive mice and challenged with *M. tuberculosis*. The adoptively immunized mice were protected, but it could not be determined whether the parenchymatous lymphocytes or the blood lymphocytes trapped in the lung capillaries were responsible. These partly inconclusive experiments led to a re-examination of the role of macrophages as mycobactericidal cells. For the sake of convenience, experiments were performed with peritoneal macrophages rather than lung macrophages. Macrophages were activated 3 weeks earlier by an intraperitoneal (IP) infection with 10^7 live BCG. The resulting macrophages were highly activated as guaged by morphology and oxidative metabolism. Yet, they exhibited no enhanced microbicidal activity *in vitro* against either mycobacterial or *Listeria* targets. However, *in vivo* IP challenge revealed enhanced (by ten-fold) inactivation of *L. monocytogenes*. There was also rapid, 1 hour, *kill* of several mycobacterial targets including two virulent strains of *M. tuberculosis*. One was hydrogen peroxide resistant, the other was susceptible. Both were killed equally well.

These experiments appear to refute the belief that mycobacteria may be intrinsically insensitive to the microbicidal mechanisms of even highly activated macrophages.

Host responses to mycobacterial infection.

In studies of non-tuberculous mycobacterial infections, mice heavily infected with some strains of *M. kansasii* or *M. avium* developed persistent infections of the lungs and spleen. The cellular defenses quickly became unresponsive to the specific mycobacterial antigens, both *in vivo* and *in vitro*. This anergic state lasts for long periods and appears to coincide with the appearance of suppressor cells within the heavily infected spleen. Despite the inability of the host to eliminate the infectious stimulus from the tissues, the cellular defenses do respond to other unrelated antigenic stimuli *in vivo*. The spleen of the tuberculin unresponsive host is in a hyperactive state, with respect to nonreplicating (sheep red blood cells) and replicating (*L. monocytogenes* and *M. tuberculosis*) antigenic stimuli. Rechallenge of these mice with an acriflavin-resistant mutant of the homologous organism revealed the presence of a population of specifically sensitized T-cells. Although they were unable to bring about a cell-mediated immune response capable of eliminating the primary bacterial population from the host tissues, they could affect the secondary challenge.

Finally, most of the reduction in mitogenic responsiveness observed when spleen cells harvested from the anergic host were exposed to PHA or PPD could be restored by the addition of an extrinsic source of the T-cell growth factor, interleukin-2. The IL-2 in the *in vitro* cultures was apparently absorbed (or destroyed) by the highly activated spleen cells also present. This arrested the cell division by the antigen-stimulated T-cells (which require IL-2 as a growth factor). Studies continue on the *in vivo* role of these cells.

Immunoregulatory mechanisms which underly anergy in tuberculosis were investigated using human blood mononuclear cell populations and *in vitro* functional assays.

Monocytes play a role in health in nonspecific suppression of lymphocyte responses to antigens and mitogens. Their suppressor activity is mediated through production of immunosuppressive prostaglandins, the action of which requires Fc receptor-bearing lymphocytes. Exposure of monocytes from healthy individuals to tuberculous plasma or mycobacterial D-arabino-D-galactan results in heightened production of prostaglandin E_2 (PGE_2) and associated increases in suppressor function. A macromolecular suppressive mediator was recently identified using a human macrophage-like cell line. This factor, molecular weight of 70,000 daltons, interferes with T-cell expression of interleukin-2 receptors and production of IL-2. Such a molecule could be important in pathophysiological states with the property of directly opposing the action of interleukin-1.

In some patients with tuberculosis, adherent cells depress tuberculin induced lymphocyte blastogenesis. This activity occurs mainly in those with skin test anergy or *in vitro* hyporesponsiveness to tuberculin antigens. Curiously, it is associated with monocyte activation. However, monocyte production of PGE_2 is not increased in tuberculosis nor is tumoricidal activity. Monocytes from tuberculous patients show altered surface expression and modulation of Class II major histocompatibility product antigens (HLA-DR), and increased production of interleukin-1. The adherent mononuclear cell responsible for suppression has been characterized as a monocyte; its activity is radiosensitive.

Some of the immunoregulatory cellular interactions important in modulating tuberculin reactivity in tuberculosis have been clarified. Future studies will be directed to identify the molecular mediator of suppression by monocytes and T-cells, particularly in relation to the basis of antigen-specificity of suppression.

Service Contracts

Serological classification of "Atypical" (Nontuberculosis) Mycobacteria: This contract is with the National Jewish Hospital/National Asthma Center, Denver, Colorado. The project deals with the so-called "atypical mycobacteria," or more preferably non-tuberculosis mycobacteria which are related to *Mycobacterium tuberculosis*. Certain of these organisms cause infections in man and animals which are clinically similar to tuberculosis. They are widespread in nature and are frequently found in soil, dust, ground water, and in some domestic water supplies. Under the auspices of the contract it was earlier established that the mycobacteria involved in many human infections can be subdivided into thirty-one serological varieties. Isolates of atypical mycobacteria received from around the world are classified (usually by serotyping) as being of a particular variety. Data of this sort are useful to epidemiologists attempting to determine the sources of these infections. In addition, the laboratory supplies materials and reagents to laboratories around the world so that they too can perform the tests. Since these materials are from a common source, they allow the

Colleagues of Dr. Goren at the console of an NMR spectrometer at the National Jewish Hospital and Research Center.

various laboratories to compare their results. Research efforts have been directed toward chemically determining the components of the bacterial cell wall that are responsible for the serological differences recognized, and this has been accomplished for many of the organisms. Chemical analysis can therefore now be used in many cases for rapid classification.

A second project is the Authentication, Maintenance, and Distribution of Mycobacterial Cultures to qualified requesters. This contract has been with the Trudeau Institute, with the National Jewish Hospital, and will in the future be held by the American Type Culture Collection. Cultures and research materials from the two projects are [illegible] used by bacteriologists, immunologists, and [illegible] [illegible] procedures in their research.

For example, during a recent contract year 398 mycobacterial strains, 78 standard [illegible] lots, and 80 non-viable mycobacterial products were supplied to qualified investigators. Some 50 strains from the Trudeau mycobacterial culture collection were propagated afresh and their identities verified. And a new species, *M.* [illegible], pathogenic for humans, was acquired for adding to the collection. The serotyping project was similarly active: nearly 1,300 cultures were received for identification. These contained 104 "spontaneous agglutinators"

which have heretofore been considered essentially untypable. However, exploitation of ELISA methodology with utilization of whole cells as antigens on one hand or crude, moderately or even purified specific glycolipid antigens, on the other, may offer the realistic prospect that this frustrating category can be eliminated.

Future goals are directed toward (1) further development of the serological typing procedure using some newly developed technologies, (2) analyzing additional chemical cell wall components, (3) further characterizing and adding recently discovered mycobacteria of pathogenic significance to the culture collection, and (4) continuing attempts to correlate the [illegible] serological variants with drug resistance and virulence.

FUTURE GOALS

The BCG field trial in South India has [illegible] [illegible] ignorance regarding the immunology of tuberculosis. And so a consensus among the members of the U.S. Tuberculosis Panel is emphasis on the support of programs [illegible] these needs. For example, what basic properties distinguish an infection caused by a virulent mycobacterium from a nonvirulent type such as BCG? Is there an intrinsic defect in the host immune system that allows the virulent organism to prevail, or does the pathogen subvert the host's defenses? Can the immune system be manipulated through immunomodulators, lymphokines, or interferons in a manner that will benefit the host?

Information is needed on the nature of the T-cell response, the expression of effector immunity, particularly in the lungs. How can a more rapid immune response be achieved? What are the precise functions of the alveolar macrophages? What mechanisms are responsible for suppression of specific lymphocyte activation?

There is need for more detailed studies on the molecular biology of mycobacteria as well as more specific diagnostic reagents of [illegible] products, antigens for immunotyping. Recombinant DNA and monoclonal antibody technology [illegible] epitopes in polypeptide antigens in *M. tuberculosis*. These may serve to improve diagnosis. Information is also urgently needed to identify antigens important in stimulating protective immunity. This relates to the antigens which activate the effector cell-mediated responses.

The emergence of Acquired Immune Deficiency Syndrome (AIDS) in the United States, and elsewhere, resulted in a surge of infections caused by members of *M. avium-intracellulare* group. **Previously** the investigation of approaches to search for mechanisms of drug resistance for "atypical" mycobacteria was considered. Now there is urgency that such studies be expanded or supported. For example, if compounds [illegible] in *M. avium* group, would it be active with all members of the group, of which mycolic acid is an integral component of the cell wall? A search [illegible] from this and other studies, possibly [illegible] should provide [illegible] in preventing [illegible] disease.

PROJECTS SUPPORTED BY THE PROGRAM

GRANTS

Investigator and Institution	*Title*
Veenna B. Antony Indiana Univ.-Purdue Univ.	Neutrophil mediated responses in pleuropulmonary tuberculosis
William W. Barrow Texas College of Osteopathic Medicine	Processing of mycobacterial lycopeptidolipid antigens
Patrick J. Brennan Colorado State University	Specific antigens of atypical mycobacteria
Alfred J. Crowle University of Colorado	Mechanisms of *M. avium* in AIDS
Thomas M. Daniel Case Western Reserve University	Specific mycobacterial antigens
Jerrold J. Ellner Case Western Reserve University	Immunosuppression in mycobacterial diseases
Pattisapu R. Gangadharam National Jewish Hospital and Research Center	Pathogenesis of *Mycobacterium intracellulare* disease
Pattisapu R. Gangadharam National Jewish Hospital and Research Center	Immunopathology of *Mycobacterium intracellulare* in AIDS
Mayer B. Goren National Jewish Hospital and Research Center	Surface chemistry of mycobacteria
Howard Gruft New York State Department of Health	Epidemiology of infection with atypical mycobacteria
Tamotsu Imaeda University of Medicine and Dentistry, NJ	*Mycobacterium avium* and *intracellulare* in AIDS patients
Maurice J. Lefford Wayne State University	Cellular defenses of the lung
Charlotte M. McCarthy New Mexico State University	Physiology of multiple drug resistant mycobacteria
Charlotte M. McCarthy New Mexico State University	Assessment of drug resistance in *Mycobacterium avium*
David N. McMurray Texas Agricultural and Mechanical University	Protein and zinc deficiency and vaccine efficacy
Henry W. Murray Cornell University	Gamma interferon in *M. avium* infections in AIDS
Quentin N. Myrvik Wake Forest University	Interaction of alveolar macrophages with mycobacteria

Thomas M. Shinnick Scrippts Clinic and Research Foundation	Reactivity of molecularly defined mycobacteria antigens
Donald W. Smith University of Wisconsin, Madison	Immunity to low virulent South Indian mycobacteria
Barnet M. Sultzer Downstate Medical Center, NY	Mitogenic activity of PPD-tuberculin

CONTRACTS

Michael D. Iseman National Jewish Hospital and Research Center	Treatment of *M. avium-intracellulare*
J. Kenneth McClatchy National Jewish Hospital and Research Center	Serological classification of atypical mycobacteria and the authentication, maintenance and distribution of mycobacterial cultures
Lowell S. Young University of California, LA	Treatment of *M. avium-intracellulare* infection in AIDS

BIBLIOGRAPHY

Alvarez, M.E., and C.M. McCarthy. Glutamine synthetase from *Mycobacterium avium*. *Can. J. Microbiol.* *30*:353–359, 1984.

Anderson, D.W., and A.J. Crowle. Evans blue dye enhances delayed hypersensitivity while blocking immunity to *Mycobacterium tuberculosis* in mice. *Infect. Immun.* *31*:413–418, 1981.

Bailey, W.C. Diagnosis of tuberculosis. *Clin. Chest Med.* *1*:209–217, 1980.

Bailey, W.C. Where do we go from here? *Am. Lung Assoc. Bull.*, pp. 21–23, 1982.

Bailey, W.C. Short-term chemotherapy of tuberculosis. *Ala. J. Med. Sci.* *20*:17–18, 1983.

Bailey, W.C., R.K. Albert, P.T. Davidson, L.S. Farer, J. Glassroth, E. Kendig, Jr., R.G. Loudon, and L.S. Inselman. Treatment of tuberculosis and other mycobacterial diseases. An official statement of the American Thoracic Society. *Am. Rev. Respir. Dis.* *127*:790–796, 1983.

Bailey, W.C. Treatment of tuberculosis. *Med. Times* *111*:16–22, 1983.

Bailey, W.C. Treatment of atypical mycobacterial disease. *Chest* *84*:625–628, 1983.

Bailey, W.C. Chemotherapy of tuberculosis. In: *Tuberculosis*. Praeger Monographs in Infectious Disease, Vol. 2. David Schlossberg, ed. New York, Praeger Publishers, pp. 65–87, 1983.

Bailey, W.C., and J.B. Bass, Jr. Treatment of tuberculosis and other mycobacterial diseases. *Am. Rev. Respir. Dis.* *129*:511, 1984.

Bailey, W.C. Clinically-derived mycobacterial classifications. *Chest* *85*:714–715, 1984.

Bailey, W.C., J.B. Bass, Jr., J.E. Hawkins, G.P. Kubica, and R.J. Wallace, Jr. Drug susceptibility testing for mycobacteria. *Am. Thoracic News* *10*:9–10, 1984.

Ballou, C.E. Specific Interaction of Mycobacterial Polymethylpolysaccharides with Long-chain Fatty Acids and Acoylcoenzyme A Derivatives. *Pure Appl. Chem.* *53*:107–112, 1982.

Band, J.D., J.I. Ward, D.W. Fraser, N.J. Peterson, V. Silcox, R.C. Good, P.R. Ostroy, and J.J. Kennedy. Peritonitis due to a *Mycobacterium chelonei*-like organism associated with intermittent chronic peritoneal dialysis. *J. Infect. Dis.* *145*:9–17, 1982.

Barksdale, L. *Mycobacterium: M. tuberculosis* and *M. bovis*. In: *Medical Microbiology*. Samuel Baron, ed., Addison Wesley Publishing Company, Chapter 38, 1985.

Barrow, W.W., B.P. Ullom, and P.J. Brennan. The peptidoglycolipid nature of the superficial cell wall sheath of smooth colony mycobacteria. *J. Bacteriol.* *144*:814–822, 1980.

Barrow, W.W., and P.J. Brennan. Isolation in high frequency of rough variants of *Mycobacterium intracellulare* devoid of C-mycoside glycopeptidolipid antigens. *J. Bacteriol.* *150*:381–384, 1982.

Barrow, W.W., and P.J. Brennan. Immunogenicity of type-specific C-mycoside glycopeptidolipids of mycobacteria. *Infect. Immun.* *36*:678–684, 1982.

Bass, J.N., P.J. Spagnuolo, and J.J. Ellner. Increased neutrophil adherence in active and remote tuberculosis. *Am. Rev. Resp. Dis.* *124*:643–645, 1981.

Benjamin, R.G., and T.M. Daniel. Serodiagnosis of tuberculosis using ELISA (enzyme linked immunoabsorbent) assay of IgG antibody to *Mycobacterium tuberculosis* antigen 5. *Am. Rev. Resp. Dis.* *126*:1013–1016, 1982.

Benjamin, R.G., S.M. Debanne, Y. Ma, and T.M. Daniel. Evaluation of mycobacterial antigens for use in enzyme-linked immunoabsorbent assay (ELISA) for the serodiagnosis of tuberculosis. *J. Med. Microbiol.*, in press.

Bennett, J.A., et al. Differences in Biological Activity among Batches of Lyophilized Tice *Bacillus Calmette-Guerin* and Their Association with Clinical Course in Stage I Lung Cancer. *Cancer Research* *43*:4183–4190, 1983.

Brennan, P.J., H. Mayer, G.O. Aspinall, and J.E. Nam Chin. Structure of glycopeptidolipid antigens from serovars in *Mycobacterium avium-Mycobacterium intracellulare-Mycobacterium scrofulaceum* (MAIS) Complex. *Europ. J. Biochem.* *115*:7–15, 1981.

Brennan, P.J., G.O. Aspinall, and J.E. Nam Shin. Structures of the specific oligosaccharides from glycopeptidolipid antigens of serovars in the *Mycobacterium avium-Mycobacterium intracellulare-Mycobacterium scrofulaceum* complex. *J. Biol. Chem.* *256*:6817–6822, 1981.

Brennan, P.J., M. Heifets, and B.P. Ullom. Thin-layer chromatography of lipid antigens as a means of identifying nontuberculous mycobacteria. *J. Clin. Microbiol.* *15*:447–455, 1982.

Brennan, P.J. Structure of the typing antigens of atypical mycobacteria—A brief review of present knowledge. *Rev. Infect. Dis.* *13*:905–913, 1981.

Brennan, P.J. Antigenic peptidoglycolipids, phospholipids and glycolipids. Chapter 18. In: *The Mycobacteria — A Sourcebook:* G.P. Kubica and L. Wayne, eds. Marcel Dekker, Inc., NY, 1984.

Brennan, P.J. New found glycolipid antigens of mycobacteria. In Microbiology, *1984.* L. Leive, and D. Schlessinger, eds. *Am. Soc. Microbiol.*, Washington, DC, 1984.

Brooks, R.W., B.C. Parker, H. Gruft, and J.O. Falkinham, III. Epidemiology of infection by nontuberculous mycobacteria. V. Numbers in eastern United States soils and correlation with soils characteristics. *Am. Rev. Resp. Dis. 130:*630-633, 1984.

Brooks, R.W., K.L. George, B.C. Parker, and J.O. Falkinham, III. Recovery and survival of nontuberculous mycobacteria under various growth and decontamination conditions. *Can. J. Microbiol.*, in press.

Carr, R.I., A.K. Chakraborty, M.J. Brunda, P.T. Davidson, P.B. Damle, M.A. Hardtke, K.J. Gilbride, and P. Minden. Immune complexes and antibodies to BCG in sera from patients with mycobacterial infections. *Clinical Exp. Immunol. 39:*562-569, 1989.

Chakraborty, A.K., P.B. Damle, P.R.J. Gangadharam, and P.T. Davidson. Studies on the interaction between 6 octylamino-5,8-quinolinequinone (CQQ) and *Mycobacterium tuberculosis. Tubercle.* 62:37-41, 1981.

Chaparas, S.D., B.W. Janicki, R. Good, A.H. Johnson, G. Wright, R. Goldstein, T.M. Daniel, and D.W. Alling. *In vitro* and *in vivo* reactivity and specificity of fractions from sonicates of *Mycobacterium tuberculosis* separated by gradient acrylamide gel electrophoresis. *Am. Rev. Resp. Dis. 122:*533-542, 1980.

Chaparas, S.D., L. Kaufman, S.J. Kim, and D.W. McLaughlin. Characterizations of antigens from *Aspergillus fumigatus.* V Reactivity in immunodiffusion tests with serums from patients with aspergillosis caused by *Aspergillus flavus, A. niger*, and *A. fumigatus. Amer. Rev. Resp. Dis. 122:*747-750, 1980.

Chaparas, S.D. Anitgenic relationship among mycobacterial species studied by modified rocket and cross immunoelectrophoresis. International Conference on Atypical Mycobacterial. *Ref. Infect. Dis. 3:*934-943, 1981.

Chaparas, S.D. Tuberculosis Immunology: Asian Pacific. *J. Allergy and Immunology 2:*128-134, 1984.

Chaparas, S.D. Clinical Mycobacteriology. Immunologically based diagnostic tests. Tuberculin and other mycobacterial antigens. In: *Source Book on Mycobacteria.* G.P. Kubica and L.G. Wayne, eds., Marcel Dekker, Inc., NY, pp. 195-220.

Chaparas, S.D. Immunological and Serological Diagnosis of Tuberculosis: State of the Art. *Indian J. Tubercul.*, 1984, in press.

Collins, F.M. Immunology of Tuberculosis. *Amer. Rev. Resp. Dis. 125:*42-49, 1982.

Collins, F.M. Cellular mediators of tuberculin hypersensitivity and antituberculous immunity. *Afr. J. Clin. Exp. Immunol. 3:*245-311, 1982.

Collins, F.M. Cellular mechanisms in antimycobacterial immunity. *Adv. Exp. Med. Biol. 162:*157-182, 1983.

Collins, F.M. Kinetics of the delayed-typed hypersensitivity response in tuberculous guinea pigs and mice tested with several mycobacterial antigens. *Amer. Rev. Resp. Dis. 127:*599-604, 1983.

Collins, F.M. Protection against mycobacterial disease by means of live vaccine tested in experimental animals. *Microbiology Series 15:*787-839, 1984.

Collins, F.M. Tuberculosis. In *Bacterial Vaccines.* R. Germainier, ed. *Academic Press*, NY, pp. 373-418, 1984.

Collins, F.M., and L.K. Auclair. Effect of *M. bovis* (BCG) infection on the kinetics of mononuclear cell response within the lung. *J. Leukocyte Biol. 35:*321-332, 1984.

Collins, F.M., and P.B. Carter. Development of delayed hypersensitivity in gnotobiotic mice. *Int. Arch. Allergy Appl. Immunol. 61:*165-174, 1980.

Collins, F.M., and D.S. Cunningham. Systemic *Mycobacterium kansasii* infection and regulation of the alloantigenic response. *Infec. Immun. 32:*614-624, 1981.

Collins, F.M., D.L. Klayman, and N.E. Morrison. Activity of 2-acetylpyridine and 2-acetylquinoline thiosemicarbazones tested *in vitro* in combination with other antituberculous drugs. *Amer. Rev. Resp. Dis. 125:*58-60, 1982.

Collins, F.M., D.L. Klayman, and N.E. Morrison. Correlations between structure and antimycobacterial activity in a series of 2-acetylpyridine thiosemicarbozones. *J. Gen. Microbiol. 128:*1349-1356, 1982.

Collins, F.M., N.E. Morrison, A.M. Dhople, and S.R. Watson. Microscopic counts carried out on *Mycobacterium leprae* and *M. tuberculosis* suspensions. A comparison of three staining procedures. *Int. J. Lep. 48:*402-407, 1980.

Collins, F.M., and L.W. Poulter. Effector and escape mechanisms in tuberculosis and leprosy. In *Immunological Aspects of Leprosy, Tuberculosis, Leishmaniasis.* D.P. Humber, ed., Excerpta Medica, Amsterdam, pp. 97-113, 1981.

Collins, F.M., and S.R. Watson. Effect of Chemotherapy on suppressor T-cells in BCG-infected mice. *Immunol. 40:*529-538, 1980.

Collins, F.M., and S.R. Watson. Immune responses to atypical Mycobacterial lung infections. *Rev. Inf. Dis. 3:*981-989, 1981.

Comstock, G.W., T.M. Daniel, D.E. Snider, Jr., P.Q. Edwards, P.C. Hopewell, and H.M. Vandiviere. The tuberculin skin test. *Am. Rev. Resp. Dis. 124:*356-363, 1981.

Crowle, A.J., and M.H. May. Preliminary demonstration of human tuberculo-immunity *in vitro*. *Infect. Immun. 31*:453–464, 1981.

Crowle, A.J., and M.H. May. Suppression of tuberculoimmunity in mice by Evans blue dye. *Infect. Immun. 38*:932-937, 1982.

Crowle, A.J., G.S. Douvas, and M.H. May. The cellular and molecular nature of human tuberculoimmunity. *Bull. Intl. Union Against Tuberc. 58*:1/72-80, 1983.

Crowle, A.J., J.A. Sharbaro, F.N. Judson, G.S. Douvas, and M.H. May. Inhibition by streptomycin of tubercle bacilli within cultured human macrophages *1-3*. *Amer. Rev. Resp. Dis.*, 1984, in press.

Crowle, A.J., and M.H. May. Replication of lyophilized and cultured BCG in human macrophages. *Amer. Rev. Resp. Dis. 128*:673–679, 1983.

Cunningham, D.S., and F.M. Collins. Suppressor and helper T-cell population in *M. kansasii*-infected spleens. *Immunol. 44*:473–480, 1981.

Cynamon, M.H., and A. Patapow. *In vitro* susceptibility of *Mycobacterium fortuitum* to Cefoxitin. *Antimicrob. Agents Chemother. 19*:205–207, 1981.

Cynamon, M.H. and G. Palmer. *In vitro* susceptibility of *Mycobacterium fortuitum* to N-formimidoyl thienamycin and several cephamycins. *Antimicrob. Agents Chemother.* 22:1079-1081, 1982.

Cynamon, M.H. and G.S. Palmer. *In vitro* susceptibility of *Mycobacterium fortuitum* to amoxicillin or cephalothin in combination with clavulanic acid. *Antimicrob. Agents Chemothe. 23*:935-937, 1983.

Cynamon, M.H. and G.S. Palmer. *In vitro* activity of amoxicillin in combination with clavulanic acid against *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother. 24*:429–431, 1983.

Daniel, T.M. The immunology of tuberculosis. *Clinics in Chest Medicine 1*:189–201, 1980.

Daniel T.M., F. van der Kuyp, and P.A. Anderson. Initial clinical trial of *Mycobacterium tuberculosis* antigen 5 in tuberculis-positive human subjects. *Am. Rev. Resp. Dis. 123*:517-520, 1981.

Daniel, T.M., M.J. Oxtoby, E. Pinto, and E. Moreno. The immune spectrum in patients with pulmonary tuberculosis. *Am. Rev. Resp. Dis. 123*:556-559, 1981.

Daniel, T.M. An immunochemists view of the epidemiology of tuberculosis. In *Prehistoric Tuberculosis in the Americas*, J.E. Buikstra, ed., Northwestern University Archeological Program, Evanston, IL, 1981.

Daniel, T.M., E.A. Balestrino, O.C. Balestrino, P.T. Davidson, S.M. Dehanne, S. Kataria, Y.P. Kataria, and J.B. Scocozza. Tuberculin specificity of *Mycobacterium tuberculosis* antigen 5. *Am. Rev. Resp. Dis. 126*:600–606, 1982.

Daniel, T.M. Robert Koch, tuberculosis, and the subsequent history of medicine. *Am. Rev. Resp. Dis. 125*(suppl.):1-3, 1982.

Daniel, T.M. Selective primary health care: Strategies for control of disease in the developing world. II. Tuberculosis. *Rev. Infect. Dis. 4*:1254-1265, 1982.

Daniel, T.M., P.A. Anderson, E.A. Balestrino, O.C. Balestrino, P.T. Davison, J.B. Scocozza, and F. van der Kuyp. The isolation, characterization and use as a tuberculin reagent of *Mycobacterium tuberculosis* antigen 5. *Bull. Internat. Union Against Tuberc. 58*:223-225, 1983.

Daniel, T.M. Tuberculosis. In *Tropical and Geographical Medicine*, K.S. Warren and A.A.F. Mahmoud, eds., McGraw-Hill, New York, pp. 786–797, 1983.

Daniel, T.M. Soluble mycobacterial antigens. In *The Mycobacteria A Sourcebook* G.P. Kubica and L.G. Wayne, eds. Marcel Dekker, Inc., New York, pp. 417–465, 1984.

Daniel, T.M. and M.E. Kleinhenz. The immunology of mycobacterial and mycotic pulmonary diseases. In *Pulmonary, Immunology and Immunologic Diseases of the Lung*, R.P. Daniele, ed., Blackwell Scientific Publication, Boston, in press.

Daniel, T.M. and G.R. Olds. Demonstration of a Shared Epitope Among Mocobacterial Antigens Using a Monoclonal Antibody. *Clin. Exp. Immun.*, in press.

Danielson, S.J. and G.R. Gray. Structures of the Two Homologous Series of Dialkene Mycolic Acids from *Mycobacterium smegmatis*. *J. Biol. Chem. 257*:12196-12203, 1982.

Dannenberg, A.M., Jr. Pathogenesis of tuberculosis. In *Pulmonary Diseases and Disorders*. A.P. Fishman, ed., McGraw-Hill Book Co., New York, pp. 1264-1281, 1980.

Dannenberg, A.M., Jr., M. Suga, and J.E. Garcia-Gonzalez. Macrophages in granulomas: Histochemical evidence suggesting local control of heterogeneous functions. In *Disorders of the Monocyte-Macrophage System*. F. Schmalzl, D. Huhn and H.E. Schaefer, eds., Berlin, Springer Verlag, pp. 109-119, 1981.

Dannenberg, A.M., Jr. Pathogenesis of pulmonary tuberculosis. *Am. Rev. Resp. Dis. 125*:(No. 3 suppl.)25–29, 1982.

Dannenberg, A.M., Jr., Pathogenesis of pulmonary tuberculosis: Basic principles. *Indian J. Chest Dis. 24*:2–3, 1982.

Dannenberg, A.M., Jr., Pathogenesis of tuberculosis: Native and acquired resistance in animals and humans. In *Microbiology, 1984*. Washington, D.C., American Society for Microbiology, pp. 344-354, 1984.

Dannenberg, A.M., Jr. Chemical and enzymatic host factors in resistance to tuberculosis. In *The Mycobacteria: A Sourcebook*. Marcel Dekker, Inc., New York, Chapter 31, pp. 721–768, 1984.

Davis, B.R., J. Brumbach, W.J. Sanders, and E. Wolinsky. Skin lesions caused by *Mycobacterium hemophilum* in a renal transplant patient. *Am. Intern. Med.* 97:723, 1982.

Dell, A. and C.E. Ballou. Fast-atombombardment, Negative-ion Mass Spectrometry of the Mycobacterial *O*-methyl-D-gluclose Polysaccharide and Lipopolysaccharides. *Carb Res. 120*:95–111, 1983.

Dell, A. and C.E. Ballou. Fast Atom Bombardment Mass Spectrometry, of a *6-O*-methylglucose Polysaccharide. *Biomed. Mass Spect. 10*:50–56, 1983.

Dhariwal, K.R., G. Dhariwal, and M. B. Goren. Observations on the ubiquity of the *Mycobacterium tuberculosis* sulfatides in mycobacteria. *Amer. Rev. Resp. Dis. 130*:641–646, 1984.

Dickinson, D.S., W.C. Bailey, B.I. Hirschowitz, S. Soong, L. Eidus, and M.M. Hodgkin. Risk factors for isoniazid (INH)-induced liver dysfunction. *J. Clin. Gastroenterol. 3*:271–279, 1981.

Edwards, M.L., D. Muller, and D.W. Smith. Influence of vaccination-challenge interval on the protective efficacy of Bacille Calmette-Guerin against low-virulence *Mycobacterium tuberculosis*. *J. Infect. Dis. 143*:739–741, 1981.

Edwards, M.L., J.M. Goodrich, D. Muller, A. Pollack, J.E. Ziegler, and D.W. Smith. Infection with *Mycobacterium avium-intracellulare* and the protective effects of Bacille Calmette Guerin. *J. Infect. Dis. 145*:733–741, 1982.

Ellner, J.J., P.J. Spagnuolo, and B.Z. Schacter. Augmentation of selective monocyte functions in tuberculosis. *J. Infect. Dis. 144*:391–398, 1981.

Ellner, J.J. Suppressor cells of man. *Clin. Immunol. Rev. 1*:119–214, 1981.

Falkinham, J.O., III, B.C. Parker, and H. Gruft. Epidemiology of infection by nontuberculous mycobacteria. I. Geographic distribution in the eastern United States. *Am. Rev. Resp. Dis. 121*:931–937, 1980.

Falkinham, J.O., III, K.L. George, B.C. Parker, and H. Gruft. Uric acid utilization by *Mycobacterium intracellulare* and *M. scrofulaceum* isolates. *J. Bacteriol. 155*:36–39, 1983.

Falkinham, J.O., III, K.L. George, B.C. Parker, and H. Gruft. *In vitro* susceptibility of human and environmental isolates of *Mycobacterium avium*, *M. intracellular* and *M. scrofulaceium* to heavy metal salts and oxyanions. *Antimicrob. Agents Chemother. 25*:137–139, 1984.

Farer, L.S. J.P. Flynn, R.J. Reza, W.C. Bailey, and R.K. Albert. Control of tuberculosis. An official statement of the American Thoracic Society. *Am. Rev. Respir, Dis. 128*:336–342, 1983.

Flatauer, F., S.P. Ballou, and E. Wolinsky. Comparative response of fever to corticosteroids in tuberculosis and in connective tissue disease. *Am. J. Med. Sci. 281*:111, 1981.

Forsberg, L.S., A. Dell, D.J. Walton, and C.E. Ballou. Revised Structure for the 6-O-methylglucose Polysaccharide of *Mycobacterium smegmatis*. *J. Biol. Chem.* 257:3555–3563, 1982.

Gangadharam, P.R.J. Atypical Mycobacterioses. *Indian J. Tubercul.* 27:108–116, 1980.

Gangadharam, P.R.J. Drug resistance and drug susceptibility tests. *Am. Rev. Resp. Dis. 122*:660–661, 1980.

Gangadharam, P.R.J., P.F. Pratt, P.B. Damle, and P.T. Davidson. Dynamic aspects of the activity of clofazimine (B663) against *Mycobacterium intracellulare*. *Tubercle*. 62:201–206, 1981.

Gangadharam, P.R.J., P.F. Pratt, and P.T. Davidson. Experimental infections with *Mycobacterium intracellulare*. *Rev. Infec. Dis. 3*:973–978, 1981.

Gangadharam, P.R.J. and A.J. Droubi. Identification of mycobacteria by smear examination of the culture. *Tubercle*. 62:123–127, 1981.

Gangadharam, P.R.J. The role of the laboratory in the management of tuberculosis patients. *Seminars Resp. Med.* 2:182–195, 1981.

Gangadharam, P.R.J. BCG vaccination and non-tuberculous mycobacteria. *Tubercle*. 62:223–224, 1981.

Gangadharam, P.R.J. and P.F. Pratt. Experimental short-course chemotherapy of *Mycobacterium kansasii* disease. *Bull. Inter. Union Against Tubercul.* 57:64–66, 1982.

Gangadharam, P.R.J., C.K. Edwards, P.S. Murthy, and P.F. Pratt. An acute infection model for *Mycobacterium intracellulare* disease using Beige mice. Preliminary results. *Amer. Rev. Resp. Dis. 127*:648–649, 1983.

Gangadharam, P.R.J. Drug parasite host interactions in tuberculosis. *Indian J. Tubercul. 30*:3–8 Special, 1983.

Gangadharam, P.R.J. and P.F. Pratt. *In vitro* response of murine alveolar and peritoneal macrophages to *Mycobacterium intracellulare*. *Amer. Rev. Resp. Dis. 128*:1044–1047, 1983.

Gangadharam, P.R.J. and P.F. Pratt. Susceptibility of *Mycobacterium intracellulare* to hydrogen peroxide. *Am. Rev Resp. Dis. 130*:309–311, 1984.

Gangadharam, P.R.J. and C.K. Edwards. Release of superoxide anion (O_2) from resident and activated mouse peritoneal macrophages infected with *Mycobacterium intracellulare*. *Am. Rev. Resp. Dis. 130*:834–838, 1984.

Gangadharam, P.R.J. Drug susceptibility or sensitivity? *Am. Rev. Resp. Dis.*, January 1985, in press.

Gangadharam, P.R.J. Animal Models for Non-tuberculosis Mycobacterial Diseases. In *Animal Models in the Evaluation of Chemotherapy of Infectious Diseases*. Merle Sande and Oto Zak, eds., Academic Press, London, 1985, in press.

Geiseler, P., K. Nelson, and R. Crispen. BCG and other measures to prevent TB in physicians. *Clinical Res. 30*:738A, 1982.

Geiseler, P., K. Nelson, and R. Crispen. Active tuberculosis in physicians. JAMA, in press.

George, K.L., B.C. Parker, H. Gruft, and J.O. Falkinham, III. Epidemiology of infection by nontuberculous mycobacteria. II Growth and survival in natural waters. *Am. Rev. Resp. Dis. 122*:89–94, 1980.

Good, R.C. Isolation of nontuberculous mycobacteria in the United States, 1979. *J. Infect. Dis. 142*:779–783, 1980.

Good, R.C., V.A. Silcox, and J.O. Kilburn. Tuberculosis and Other Mycobacterioses. In *Diagnostic Procedures for Bacterial, Mycotic, and Parasitic Infections*, A. Balows and W.J. Hausler, Jr., eds., American Public Health Association, Washington, D.C., pp. 675-703, 1981.

Good, R.C. and D.E. Snider, Jr. Isolation of nontuberculous mycobacteria in the United States, 1980. *J. Infect. Dis. 146*:829-833, 1982.

Good, R.C. and R.E. Bernam. Seroagglutination. In *The Mycobacteria: A Sourcebook*, G.P. Kubica and L.G. Wayne, Eds., Marcel Dekker, Inc., New York and Basel, pp. 105-122, 1984.

Good, R.C. Diseases in nonhuman primates. In *Mycobacteria: A Sourcebook*, G.P. Kubica and L.G. Wayne, eds., Marcel Dekker, Inc., New York and Basel, pp. 903-924, 1984.

Goodfellow, M. and L.G. Wayne. Taxonomy and Nomenclature. In *The Biology of the Mycobacteria*. C. Ratledge and J. Stanford, eds., London Academic Press, Vol. 1, pp. 471–521, 1982.

Goren, M.B., D.L. Swendsen, and J. Henson. Factors modifying the fusion of phagosomes and lysosomes: art, fact and artefact. In *Mononuclear Phagocytes-Functional Aspects*, R. van Furth, ed., Martinus-Nijhoff, The Hague, 1980.

Goren, M.B. and K.-S. Jiang. α-D-glucopyranuronosyl (1–1) α-D-glucopyranuronoside and simple derivatives. *Carbohydrate Res. 79*:225–234, 1980.

Goren, M.B. Immunoreactive substances of mycobacteria. *Amer. Rev. Resp. Dis. 125*:50–69, 1982.

Goren, M.B. Some paradoxes of macrophage function. In *Host Defenses to Intracellular Pathogens*: T. Eisenstein, P. Actor, and H. Friedman, eds., Plenum, New York, 1983.

Goren, M.B., J.M. Grange, V. R. Aber, B.W. Allen, and D.A. Mitchison. Lipid content, guinea pig virulence, bacteriophage typing and associated characteristics of *M. tuberculosis*. *British J. Exp. Pathol. 63*:693–700, 1982.

Goren, M.B., C.L. Swendsen, J. Fiscus and C. Miranti. Fluorescent markers for studying phagosome-lysosome fusion. *J. Leukocyte Biol. 36*:273–292, 1984.

Goren, M.B. Biosynthesis and structures of phospholipds and sulfatides. In *The Mycobacteria: A Source Book*: G. Kubica and L. Wayne, ed., Marcel Dekker, New York, 1984.

Gray, G.R., M.Y.H. Wong, and S.J. Danielson. The Major Mycolic Acids of *Mycobacterium smegmatis*. *Progress in Lipid Research, 21*:91–107, 1983.

Greene, J.B., et al. *Mycobacterium avium-introcellulare*: A cause of disseminated life-threatening infection of homosexuals and drug abusers. *Ann. Intern. Med. 97*:539–546, 1982.

Gruft, H. Sensitivity testing of *Mycobacterium tuberculosis*. *New York State J. Med. 81*:1022, 1981.

Gruft, H., R. Johnson, R. Claflin, and A. Loder. The use of phage typing and drug resistance as a tool in mycobacterial epidemiology. *Am. Rev. Resp. Dis. 130*:96–97, 1984.

Gruft, H., J.O. Falkinham III, B.C. Parker. Recent experience in the epidemiology of nontuberculous mycobacterial diseases. *Rev. Infect. Dis. 3*:990–996, 1981.

Hall, M.R. and C.O. Thoen. Production and evaluation of lysozyme extracts of virulent *Mycobacterium bovis*. *Am. J. Vet. Res.*, 1985, in press.

Hank, J.A., J.K. Chan, M.L. Edwards, D. Muller, and D.W. Smith. Influence of the virulence of *Mycobacterium tuberculosis* on protection induced by Bacille Calmette-Guerin in guinea pigs. *J. Infect. Dis. 143*:734–738, 1981.

Hawkins, J.E., R.C. Good, G.P. Kubica, P.R.J. Gangadharam, H.M. Gruft, K.D. Stottmeier, H.M. Sommers, and L.G. Wayne. Levels of laboratory service of mycobacterial diseases. Official statement of the American Thoracic Society *Am. Rev. Resp. Dis. 128*:213–218, 1983.

Hawkins, J.E., R.C. Good, G.P. Kubica, P.R.J. Gangadharam, H.M. Gruft, K.D. Stottmeier, H.M. Sommers, and L.G. Wayne. Mycobacteriology Laboratories. *Am. Rev. Resp. Dis. 128*:957, 1983.

Hawkins, J.E., R.C. Good, G.P. Kubica, P.R.J. Gangadharam, and H.M. Gruft. The levels of service concept in mycobacteriology. *Amer. Thor. Soc. News* 9:19–25, 1983.

Hawkins, J.E., R.C. Good, G.P. Kubica, P.R.J. Gangadharam, H.M. Gruft, and K.D. Stottmeier. Levels of laboratory service for mycobacterial diseases. Official statement of the American Thoracic Society. *Am. Rev. Resp. Dis. 128*:213, 1983.

Heifets, L., K. Imai, and M.B. Goren. Expression of peroxidase-dependent iodination by macrophages ingesting neutrophil debris. *J. Reticuloendothel. Soc. 28*:391–404, 1980.

Hepper, K.P. and F.M. Collins. Immune responsiveness in mice heavily infected with *Mycobacterium kansasii. Immunol. 53*:357–365, 1984.

Hepper, K.P. and F.M. Collins. Immune responsiveress in mice heavily infected with *Mycobacterium kansasii. Immunol. 53*:357–365, 1984.

Hepper, K.P. and F.M. Collins. Adoptive transfer of acquired resistance to *Mycobacterium kansasii* infection by splenic T-cells harvested from chronically infected mice. *Immunol. 53*: 1984, in press.

Higuchi, S., A.M. Dannenberg, L.F. Affronti, I. Azuma, T.M. Daniel, L.A. Sternberger, and J.P. Petrali. Persistance of protein carbohydrate and wax components of tubercle bacilli in primary BCG lesions and those of reinfection. *Am. Rev. Resp. Dis. 123*:397–401, 1981.

Higuchi, S., M. Suga, A.M. Dannenberg, Jr., L.F. Affronti, I. Azuma, T.M. Daniel, and J.P. Petrali. Persistance of protein, carbohydrate and wax components of tubercle bacilli in dermal BCG lesions. *Am. Rev. Resp. Dis. 123*:397–401, 1981.

Hindsgaul, O. and C.E. Ballou. Affinity Purification of Mycobacterial Polymethyl Polysaccharides and a Study of Polysaccharide-Lipid Interactions by ^{1}H NMR. *Biochemistry 23*:577–584, 1984.

Hon, J.K., R.W. Hamner, W.C. Bailey, and T.W. Sheehy. Metastatic melanoma complicated by disseminated BCG. *Sou. Med. J. 75*:1263–1264, 1982.

Horsburgh, C.R., Jr. and C.H. Kirkpatrick. A microtiter assay for human monocyte activation by lymphokines. *J. Immunol. Meth. 72*:207–217, 1984.

Huang, H.C., J.K. Rehmann, and G.R. Gray. Total Synthesis of Naturally-Occurring Mycolic Acids (*E*)-and (*Z*)-*threo*-2-Docosyl-3-hydroxytetracont-21-enoate. *J. Org. Chem. 47*:4018–4023, 1982.

Hunter, S.W., R.C. Murphy, K. Clay, M.B. Goren, and P.J. Brennan. Trehalose-containing lipooligosaccharides: A new class of species-specific antigens from *Mycobacterium. J. Biol. Chem. 258*:10481–10487, 1983.

Hunter, S.W., T. Fujiwara, R.C. Murphy, and P.J. Brennan. N-acyl-kansosamine: a novel N-acylaminosugar from the trehalose-containing lipooligosaccharide of antigens of *Mycobacterium kansasii. J. Biol. Chem. 259*:9729–9734, 1984.

Hunter, S.W., I. Jardine, D.L. Yanagihara, and P.J. Brennan. The trehalose-containing lipooligosaccharides from mycobacteria: Structures of the oligosaccharide segments and recognition of a unique N-acylkansosamine-containing epitope. *Biochemistry*. In press.

Imaeda, T. Cell wall deficient forms. In *The Mycobacteria*, G.P. Kubica and L.G. Wayne, eds., pp. 671–680, Marcel Dekker, Inc., NY, 1984.

Johnson, A.H., G.L. Wright, S.D. Chaparas, and D.J. Mancuso. Analytical gradient polyacrylamide gel crossed line immunoelectrophoresis. A technique for localizing *Mycobacterium tuberculosis* H37Rv antigen in polyacrylamide gel columns. *Immunol. Comm.* 9(6):595–609, 1980.

Jones, W.D., Jr., R.C. Good, N.J. Thompson, and G.D. Kelly. Bacteriophage types of *Mycobacterium tuberculosis* in the United States. *Am. Rev. Resp. Dis. 125*:640–643, 1982.

Kaplan, M.H., and M.H. Chase. Antibodies to mycobacterium in human tuberculosis, I. Development of antibodies before and after antimicrobial therapy. *J. Infect. Dis. 142*:825–834, 1980.

Kaplan, M.H., and M.H. Chase. Antibodies to mycobacterium in human tuberculosis, II. Responses to nine defined mycobacterium antigens giving evidence for antibody common to tuberculosis and lepromatous leprosy. *J. Infect. Dis. 142*:835–843, 1980.

Kasik, J.E., M. Monick, and B. Schwarz. β-Lactamase Activity in Slow-Growing Nonpigmented Mycobacteria and Their Sensitivity to Certain β-Lactam Antibiotics. *Tubercle 61*:213, 1980.

Kasik, J.E., and M. Monick. A Comparison of Antibacterial and Anti-immune Effects of Certain Rifamycins. *Antimicrob. Ag. and Chemother. 19*:134, 1981.

Kasik, J.E. Central Nervous System Tuberculosis. In *Tuberculosis. Praeger Monographs in Infectious Disease*, David Schlossberg, ed., 2:141–157, 1983.

Kasik, J.E. Tuberculosis and Other Mycobacterial Disease. In *Current Therapy*, H.F. Conn, ed., Uniontown, PA, pp. 125–132, 1984.

Katoch, V.M., L.G. Wayne, and G.A. Diaz. Serological approaches for the characterization of catalase in tissue derived mycobacteria. *Ann. Microbiol. (Inst. Pasteur) 133B*:407–414, 1982.

Katoch, V.M., L.G. Wayne, and G.A. Diaz. Characterization of catalase by micro-immunoprecipitation in tissue-derived cells of *M. lepraemurium* TMC 1701. *Int. J. Syst. Bacter. 32*:416–418, 1982.

Kilburn, J.O., K. Takayama, E.L. Armstrong, and J. Greenberg. Effects of Ethanbutol on Phospholipid Metabolisms in *Mycobacterium smegmatis*. *Antimicrob. Ag. Chemother.* *19*:346–348, 1981.

Kilburn, J.O., and K. Takayama. Effects of Ethanbutol on Accumulation and Secretion of Trehalose Mycol and Free Mycolic Acid in *Mycobacterium smegmatis*. *Antimicrob. Ag. Chemother.* *20*:401–404, 1981.

Kilburn, J.O., K. Takayama, and E.L. Armstrong. Synthesis of Trehalose Dimycolate (Cord Factor) by a Cell-Free System of *Mycobacterium smegmatis*. *Biochem. Biophys. Res. Commun.* *108*:132–139, 1982.

Kleinhenz, M.E., J.J. Ellner, P.J. Spagnuolo, and T.M. Daniel. Suppression of lymphocyte responses by tuberculous plasma and myochacterial arabinogalactan: monocyte dependence and indomethacin reversibility. *J. Clin. Invest.* *68*:153–162, 1981.

Kleinhenz, M.E., and J.J. Ellner. Divergent T cell functions in antigen-induced blastogenesis: facilitory interactions with Tnon cells and participation in monocyte- and prostaglandin-mediated suppression. *J. Lab. Clin. Med.* *102*:751–761, 1983.

Kubica, G.P., and R.C. Good. The Genus *Mycobacterium* (except *M. leprae*). In *The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria*, M.P. Starr, H. Stolp, H.G. Truper, A. Balows and H.G. Schlegel, eds., Springer-Verlag, Heidelberg, pp. 1962–1984, 1981.

Kubica, G.P. Correlation of acid-fast staining methods with culture results for mycobacteria. *Bull. Int. Un. Tuberc.* *55*:117–124, 1980.

Kubica, G.P. Phage typing of *Mycobacterium tuberculosis* - a time for standardization. *Am. Rev. Resp. Dis.* *126*:3–4, 1982.

Kubica, G.P. Clinical Microbiology. In *The Mycoabacteria: A Sourcebook*, Marcel Dekker, Inc., NY, Chpt. 7, pp. 133–176, 1984.

Kuritsky, J.N., M.G. Bullen, C.V. Broome, V.A. Silcox, R.C. Good, and R.J. Wallace, Jr. Sternal wound infections and endocarditis due to organisms of the *Mycobacterium fortuitum* complex. *Ann. Intern. Med.* *98*:938–939, 1983.

Leake, E.S., Q.N. Myrvik, and M.J. Wright. Phagosomal membranes of *Mycobacterium bovis* BCG-immune alveolar macrophages are resistant to disruption by *Mycobacterium tuberculosis* H37Rv, *Infect. Immun.* *45*:443–446, 1984.

Liav, A., and M.B. Goren. Synthesis of 6,6′-o a trehaloses via hexa-O-benzyl trehalose. *Carbohydrate Res.* *81*:C1–C3, 1980.

Liav, A., and M.B. Goren. An improved synthesis of hexa-O-acetyl (α-D-glucopyranosyluronic acid) (α-D-glucopyranosiduronic acid). *Carbohydrate Res.* *84*:171–174, 1980.

Liav, A., and M.B. Goren. A new synthesis of cord factors and analogs. *Chem. Phys. Lipids* *27*:345–352, 1980.

Liav, A., and M.B. Goren. 6,6′-Diamino-2,3,4,2′3′,4′-hexa-O-benzyl-6,6′-dideoxy-α,α-trehalose. *Carbohydrate Res.* *87*:153–155, 1980.

Liav, A., and M.B. Goren. Concerning 2,3,4,2′3′,4′ hexa-O-acetyl-6,6′-diamino-6,6′-dideoxy-α,α-trehalose. *Carbohydrate Res.* *87*:289–295, 1980.

Liav, A., B.C. Das, and M.B. Goren. Diamide pseudo cord factors: bis-N-acyl derivatives of 6,6′-diamino-6,6′-dideoxy-α,α-trehalose. *Carbohydrate Res.* *94*:230–235, 1981.

Liav, A., and M.B. Goren. 3′, 6′-anhydro-6-0-coryno-mycoloyl trehalose: Synthesis and identification as the impurity in synthetic cord factor preparations. *Chem. Phys. Lipids* *30*:27–34, 1982.

Liav, A., and M.B. Goren. Synthesis of α-D-mannopyranosyl α-D-mannopyranoside from α,α-trehalose: a route to cord factor analogs. *Carbohydrate Res.* *123*:C22–C24, 1983.

Liav, A., and M.B. Goren. Synthesis of 6-0-mycoloyl and 6-0-corynomycoloyl-α,α-trehalose. *Carbohydrate Res.* *125*:323–328, 1984.

Liav, A., and M.B. Goren. Sulfatides of *Mycobacterium tuberculosis*. Synthesis of the core α,α-trehalose 2-sulfate. *Carbohydrate Res.* *127*:211–216, 1984.

Liav, A., and M.B. Goren. Sulfate as a blocking group in a alkali-catalyzed permethylation: an alternative synthesis of a 3,4,6-tri-O-methyl-D-glucose. *Carbohydrate Res.* *131*:C8–C10, 1984.

Liav, A., and M.B. Goren. Cord-Factor Analogs: Synthesis of 6,6′-Di-O-Mycoloyl- and -Corynomycoloyl-α-D-Mannopyranosyl α-D-Mannopyranoside. *Carbohydrate Res* *129*:121–129, 1984.

Liav, A., H.M. Flowers, and M.B. Goren. Cord Factor Analogs: Synthesis of 6,6′-Di-O-Mycoloyl – and – corynomycology (α-D- galactopyranosyl α-D – galactopyranoside). *Carbohydr. Res.*, *133*:53–58, 1984

Linscheid, M., J. D'Angona, A.L. Burlingame, A. Dell, and C.E. Ballou. Field Desorption Mass Spectrometry of Oligosaccharides. *Proc. Natl. Acad. Sci. USA* *78*:1471–1475, 1981.

Ma, Y., and T. M. Daniel. Immunochemical analysis of tuberculin purified protein derivative with special reference to United States-Japan antigen 7. *J. Infect. Dis.* *148*:500–509, 1983.

Maloney, D.H., and C.E. Ballou. Polymethylpolysaccharide Synthesis in an Ethionine-resistant Mutant of *Mycobacterium smegmatis* *J. Bacteriol.* *141*:1217–1221, 1980.

Mason, U.G., L.E. Greenberg, S.S. Yen, and C.H. Kirkpatrick. Indomethacin-responsive mononuclear cell dysfunction in "atypical" mycobacteriosis. *Cell Immunol 71*:54-65, 1982.

Mason, U.G., and C.H. Kirkpatrick. Modulation of immunologic responses in nontuberculous mycobacterial infections with indomethacin. *J. Clin. Immunol. 4*:112-117, 1984.

Mason, U.G, L. Greenberg, S. Yen, and C.H. Kirkpatrick. Improvement of abnormal lymphocyte responses in "atypical" mycobacterioses with indomethacin. In *Host Defenses to Intracellular Pathogens*. T. Eisenstein, P. Actor and H. Friedman, eds., Plenum Press, NY, pp. 197-201, 1983.

Mason, U.G., and C.H. Kirkpatrick. The lymphoid system in mycotic and mycobacterial infections. In *Advances in Host Defense Mechanisms*, J.I. Gallin and A.S. Fauci, eds., Raven Press, NY, 2:143-177, 1983.

Mathews, H.L., J. Brenman, P.J. Kelleher, and P. Minden. Suppression of the line-10 guinea pig hepatocarcinoma by antigens derived from *Mycobacterium bovis* (BCG) that are common to the tumor. *J. Biol. Response Modifiers 1*:253-265, 1982.

McCarthy, C., and P. Ashbaugh. Factors that affect the cell cycle of *Mycobacterium avium. Rev. Infect. Dis. 3*:914-925, 1981.

McCarthy, C. Variation in isoniazid susceptibility of *Mycobacterium avium* during the cell cycle. *Am. Rev. Resp. Dis. 123*:450-453, 1981.

McCarthy, C. Continuous Culture of *Mycobacterium avium* limited for ammonia. *Am. Rev. Resp. Dis. 127*:193-197, 1983.

McCarthy, C. Free fatty acid and triglyceride content of *Mycobacterium avium* cultured under different growth conditions. *Am. Rev. Resp. Dis. 129*:96-100, 1984.

McGee, Maria P., Q. N. Myrvik, B. Y. Thompson, L. J. Eaton, and J. Ockers. Macrophage Heterogeneity in BCG-Induced Granulomas. In *Basic and Clinical Aspects of Granulomatous Diseases*. D.L. Boros and T. Yoshida, eds., pp. 51-83, Elsevier North Holland Publishers, NY, 1980.

McMurray, D.N. Mechanisms of anergy in tuberculosis. *Chest 77*:4-5, 1980.

McMurray, D.N., E.A. Yetley, and T. Burch. Effect of malnutrition and BCG vaccination on macrophage activation in guinea pigs. *Nutr. Res. 1*:373-384, 1981.

McMurray, D.N., and E.A. Yetley. Immune responses in malnourished guinea pigs. *J. Nutr. 112*:167-174, 1982.

McMurray, D.N., and E.A. Yetley. Cell-mediated immunity in malnourished guinea pigs after *Mycobacterium bovis* BCG vaccination. *Infect. Immun. 35*:909-914, 1982.

McMurray, D.N., M.A. Carlomagno, and P.A. Cumberland. Respiratory infection with attenuated *Mycobacterium tuberculosis* H37Ra in malnourished guinea pigs. *Infect. Immun. 39*:793-799, 1983.

McMurray, D.N., and E.A. Yetley. Response to *Mycobacterium bovis* BCG vaccination in protein- and zinc-deficient guinea pigs. *Infect. Immun. 39*: 1983.

McMurray, D.N. Cell-mediated immunity in nutritional deficiency. In *Progress in Food and Nutrition Science*, Vol. 8. R.K. Chandra, ed., Pergamon Press, NY, 1984, in press.

Meissner, P.S., and J.O. Falkinham III. Plasmid-encoded mercuric reductase in *Mycobacterium scrofulaceum. J. Bacteriol. 157*:669-672, 1984.

Merkal, R.S., W.G. McCullough, and K. Takayama. Mycobactins, the State of the Art. *Bulletin de L'Institut Pasteur 79*:251-259, 1981.

Minden, P., L.F. Odon, D.G. Tubergen, M.A. Hardtke, T.R. Sharpton, B. Rose, A. Zlotnick, and R.T. Carr. Immune complexes in children with leukemia: Relationship to disease characteristics and to antibody response to *Mycobacterium bovis* (BCG) in patients receiving BCG immunotherapy. *Cancer 45*:460-468, 1980.

Minden, P., and H.L. Mathews. Suppression and immunotherapy of the guinea pig line-10 hepatocarcinoma mediated by heat-killed disrupted *Mycobacterium bovis* (BCG). *Cancer Research 40*:3214-3217, 1980.

Minden, P., H.L. Mathews, and P.J. Kelleher. Suppression of the line-10 guinea pig hepatocarcinoma by antigens related to both *Mycobacterium bovis* (BCG) and the tumor. *J. Immunol. 125*:2685-2689, 1980.

Minden, P., P. Kelleher, and L.K. Woods. The association between antigens in human malignant melanoma cells and *Mycobacterium bovis* (BCG). In *Melanoma Antigens and Antibodies*, Chapter 14. R.A. Reisfeld and S. Ferrone, eds., Plenum Press, NY, 1982.

Minden, P., H.L. Mathews, and P.J. Kelleher. Biological significance of antigens shared by line-10 hepatocarcinoma cells and *Mycobacterium bovis* (BCG). In *Cancer: Etiology and Prevention*. R.G. Crispen, ed., Elsevier Science Publishing Co., Inc., NY, 1983.

Minden, P., P.J. Kelleher, J.H. Freed, L.D. Nielsen, P.J. Brennan, L. McPheron, and J.K. McClatchy. Immunological evaluation of a component isolated from *Mycobacterium bovis*, strain BCG using a monoclonal antibody to BCG. *Infect. Immun.*, Nov., 1984.

Myrvik, Q.N. Immunopathology of Pulmonary Granulomas. In *Macrophages and Natural Killer Cells*: S. Normann and E. Sorkin, eds., Plenum Press, pp. 649-657, 1982.

Myrvik, Q.N., E.S. Leake, and K.T. Racz. Interaction of mycobacteria with normal and immunologically-activated macrophages. In *Host Defenses to Intracellular Pathogens*. T.K. Eisenstein, P. Actor and H. Friedman, eds., Plenum Press, NY, 1983.

Myrvik, Q.N., E.S. Leake, and M.J. Wright. Disruption of phagosomal membranes of normal alveolar macrophages by the H37Rv strain of *Mycobacterium tuberculosis*: A correlate of virulence. *Am. Rev. Resp. Dis. 129*:322-328, 1984.

Namba, M., A.M. Dannenberg, Jr., and F. Tanaka. Improvement of the histochemical demonstration of acid phosphatase, B-galactosidase and nonspecific esterase in glycol methacrylate tissue sections by cold temperature embedding. *Stain Technol. 58*:207-213, 1983.

Namba, M., M. Suga, F. Tanaka, A.M. Dannenberg, Jr., A.T. Hastie, and R.C. Franson. Immunocytochemical demonstration of rabbit ribonuclease and phospholipase A_2 by the peroxidase-antiperoxidase technique in professional phagocytes (pulmonary alveolar macrophages and granulocytic and mononuclear peritoneal exudate cells) and in glycol methacrylate sections of dermal tuberculous (BCG) lesions. *J. Reticuloendothel. Soc. 34*:425-435, 1983.

Norgard, M.V., and T. Imaeda. Heterogeneity of deoxyribonucleic acid molecules isolated from *Mycobacterium smegmatis. J. Bacteriol. 144*:766-771, 1980.

Orme, I.M., and F.M. Collins. Resistance of various strains of Mycobacteria to killing by activated macrophages *in vivo*. *J. Immunol. 131*:1452-1454, 1983.

Orme, I. M., and F.M. Collins. Protection against *Mycobacterium tuberculosis* infection by adoptive immunotherapy. *J. Exp. Med. 158*:74-83, 1983.

Orme, I.M., and F.M. Collins. Infection with *M. kansasii* and efficiency of vaccination against tuberculosis. *Immunol. 50*:581-586, 1984.

Orme, I.M., and F.M. Collins. The immune response to atypical Mycobacteria. Immunocompetence of heavily infected mice measured *in vivo* fails to substantiate immunosuppression data obtained *in vitro*. *Infect. Immun. 43*:31-37, 1984.

Orme, I.M., and F.M. Collins. Efficacy of BCG vaccination in mice undergoing prior pulmonary infection with atypical Mycochacteria. *Infect. Immun. 44*:28-32, 1984.

Orme, I.M., and F.M. Collins. Demonstration of acquired resistance in *Bcg*ʳ inbred mouse strains infected with a low dose of BCG Montreal. *Clin. Exp. Immunol. 56*:81-88, 1984.

Orme, I.M., and F.M. Collins. Adoptive protection of the *M. tuberculosis*-infecting lung. Dissociation between cells that passively transfer protective immunity and those that transfer delayed-type hypersensitivity to tuberculin. *Cell Immunol.*, 1984, in press.

Orme, I.M., M.J.H. Ratcliffe, and F.M. Collins. Acquired immunity to heavy infection with *Mycobacterium bovis* (BCG) and its relationship to the development of non-specific unresponsiveness *in vitro*. *Cell. Immunol.*, 1984, in press.

Parker, B.C., M.A. Prod, H. Gruft, and J.O. Falkinham, III. Epidemiology of infection by nontuberculous mycobacteria. IV. Preferential aerosolization of *Mycobacterium intracellulare* from natural waters. *Am. Rev. Respr. Dis. 128*:652-656, 1983.

Payne, V.D.N., and T.M. Daniel. Identification and preliminary purification of a protein antigen apparently specific to *Mycobacterium kansasii*. *Am. Rev. Resp. Dis. 122*:311-317, 1980.

Qureshi, N., K. Takayama, and H.K. Schnoes. Purification of C_{30-56} Fatty Acids from *Mycobacterium tuberculosis* H37Ra. *J. Biol. Chem.* 255:182-189, 1980.

Qureshi, N., K. Takayama, and H.K. Schnoes. Separation of a Homologous series of Long-Chain Fatty Alcohols (C_{60}–C_{88}) from *Mycobacterium tuberculosis* H37Ra by High Performance Liquid Chromatography. *J. Liquid Chromatogra.* 4:1207-1218, 1981.

Qureshi, N., N. Sathyamoorthy, and K. Takayama. Biosynthesis of C_{30} to C_{56} Fatty Acids by an Extract of *Mycobacterium tuberculosis* H37Ra. *J. Bacteriol. 157*:46-52, 1984.

Retzinger, G.S., S.C. Meredith, K. Takayama, R.L. Hunter, and F.J. Kezdy. The Role of Surface in the Biological Activities of Trehalose 6,6′-Dimycolate. Surface Properties and Development of a Model System. *J. Biol. Chem.* 256:8208-8216, 1981.

Retzinger, G.S. S.C. Meredith, R.L. Hunter, K. Takayama, and F.J. Kezdy. Identification of the Physiologically Active State of the Mycobacterial Glycolipid Trehalose 6,6′-Dimycolate and the Role of Fibrinogen in the Biological Activities of Trehalose 6,6′-Dimycolate Monolayers. *J. Immunol. 129*:735-744, 1982.

Retzinger, G.S., S.C. Meredith, R.L. Hunter, K. Takayama, and F.J. Kezdy. Identification of the physiologically active state of the mycobacterial glycolipid trehalose 6, 6′ dimycolate and the role of fibrinogen in the biologic activities of trehalose 6, 6′ dimyoclate monolayers. *J. Immunol. 129*:735, 1982.

Runyon, E.H., A.G. Karlson, G.P. Kubica, and L.G. Wayne. Mycobacterium. In, *Manual of Clinical Microbiology, 3rd edition*. E.H. Lennette, A. Balows, W.J. Hausler, and J.P. Truant, eds., American Society of Microbiology, Washington, DC, 1980.

Saadat, S., and C.E. Ballou. Pyruvylated Glycolipids from *Mycobacterium smegmatis*. Structure of two oligosaccharide components. *J. Biol. Chem.* 258:1813-1818, 1982.

Saadat, S., and C.E. Ballou. Separation of O-methylhexitols and O-methylmyo-inositols by Reverse-phase High-performance Liquid Chromatography. *Carb. Res. 119*:248–253, 1983.

Sanders, W.J., and E. Wolinsky. *In vitro* susceptibility of *Mycobacterium marinum* to eight antimocrobial agents. *Antimicrob. Agents Chemother. 18*:529, 1980.

Sathyamoorthy, N., N. Qureshi, and K. Takayama. Purification and Characterization of C_{28-35} Fatty Acids from *Mycobacterium smegmatis*. *Can. J. Microbiol.*, in press.

Schroff, R.W., E.R. Heise, and Q.N. Myrvik. Characterization of the Early Phase of the Tuberculin Reactions in Rabbits. *Am. Rev. Respir. Dis. 121*:843–850, 1980.

Schroff, R.W., E.R. Heise, Q.N. Myrvik, and B.T. Shannon Selective Suppression of Granuloma Formation and Delayed Hypersensitivity in Rabbits. *Infect. Immun. 28*:269–276, 1980.

Seggev, J., M.B. Goren, R.I. Carr, and C.H. Kirkpatrick. Interstitial and hemorrhagic pneumonitis induced by mycobacterial trehalose dimycolate. *Am. J. Path. 106*:348–355, 1982.

Seggev, J.S., C.H. Kirkpatrick, and M.B. Goren. The pathogenesis of interstitial pneumonitis induced by trehalose dimycolate II. Reserpine prevents formation of lesions. *Am. Rev. Resp. Dis. 129*:840–843, 1984.

Seggev, J.S., M.B. Goren, and C.H. Kirkpatrick. The pathogenesis of trehalose dimycolate-induced interstitial pneumonitis. III. Evidence for a role for T-lymphocytes. *Cell Immunol. 85*:428–435, 1984.

Shively, J., J.G. Songer, S. Pruchal, M. Keasey, and C.O. Thoen. *Mycobacterium kansasii infection in bufonidae. J. Wildlife Dis. 17*:3–7, 1981.

Siddiqi, S.H., C.C. Hwangbo, V. Silcox, R.C. Good, D.E. Snider, Jr., and G. Middlebrook. Rapid radiometric methods to detect and differentiate *Mycobaterium tuberculosis, M. bovis* from other Mycobacterial species. *Am. Rev. Resp. Dis. 130*:634–640, 1984.

Smith, D.W. Mycobacteria. In *Medical Microbiology and Infectious Diseases*. A.I. Braude, ed., W.B. Saunders Company, 1981.

Smith, D.W. Koch's postulates and the Koch phenomenon—an update. *Indian J. Chest Dis. 24*:95–100, 1982.

Smith, D.W., A. Ladefoged, P. Reeser, and M. Edwards. Does trypsin extract soluble antigen meet the criteria of immunogenicity without allergenicity? *Tubercle 65*:105–110, 1984.

Smith, D.W. Protective effect of BCG in experimental tuberculosis. *Adv. Tuberc. Res.*, in press.

Smith, D.W. BCG vaccination. In *Mycobacteria, A Sourcebook*, G. Kubica and L. Wayne, eds., Marcel Dekker, Inc., 1984.

Smith, D.W. Tuberculosis in guinea pigs. In *Mycobacteria, A Sourcebook*, G. Kubica and L. Wayne, eds., Marcel Dekker, Inc., 1984.

Smith, D.W., P. Reeser, and S. Musa. Does infection with environmental mycobacteria suppress the protective response to subsequent vaccination with BCG? *Tubercle*, in press.

Snider, D.E., Jr., W.D. Jones, Jr., and R.C. Good. The usefulness of phage typing *Mycobacterium tuberculosis* isolates. *Am. Rev. Resp. Dis.*, in press.

Sopko, J.A., J. Fieselmann, and J.E. Kasik. Pulmonary Disease Due to Mycobacterium Chelonei Subspecies Abscesses: A Report of Four Cases. *Tubercle 61*:165, 1980.

Stroebel, A.B., T.M. Daniel, J.H. K. Lau, J.C. Y. Leong, and H. Richardson. Serological diagnosis of bone and joint tuberculosis by an ELISA assay. *J. Infect. Dis. 146*:280–283, 1982.

Suga, M., A.M. Dannenberg, Jr., and S. Higuchi. Macrophage functional heterogeneity in vivo: macrolocal and microlocal macrophage activation, identified by double-staining tissue sections of BCG granulomas for pairs of enmzymes. *Am. J. Pathol. 99*:305–324, 1980.

Takayama, K., H.C. Jordi, and F. Benson. Separation of Fatty Acids as their p-bromophenacyl Esters on a C_{18}- Bonded Silica Column by High Performance Liquid Chromotography. *J. Liquid Chromatogr. 3*:61–69, 1980.

Takayama, K. N. Qureshi, E. Ribi, J.L. Cantrell, and K. Amano. Use of Endotoxin in Cancer immunotherapy and Characterization of its Non-toxic but Active Lipid A Components. In *Bacteriol. Lipopolisaccharide, Structure, Synthesis and Biological Activities*. L. Anderson and F.M. Unger, eds. ACS Symposium Series 231. American Chemical Society, Washington, DC, pp. 219–233, 1983.

Takayama, K. and N. Qureshi. Structure and Synthesis of Lipids. In *The Mycobacteria, A Sourcebook, Part A.*, G. P. Kubica and L. G. Wayne, eds. Marcel Dekker, Inc., NY, pp. 315–344, 1984.

Thoen, C.O. Mycobacterium. In *Diagnostic Procedures in Veterinary Microbiology, 4th edition*. G. R. Carter, ed., C. C. Thomas Publisher, Springfield, IL, pp. 219–228, 1984.

Thoen, C.O. and T. Schiesser. Mycobacterial Disease in Cold-blooded Animals. in *The Mycobacterial: A Sourcebook*. G.P., Kubica and L.G. Wayne, ed., Marcel Dekker, Inc., NY, pp. 1297–1309, 1984.

Thoen, C.O., A.G. Karlson, and E.M. Himes. Mycobacterium tuberculosis Complex Infections in Animals. In *The Mycobacteria: A Sourcebook*. G.P. Kubica and L.G. Wayne, ed., Marcel Dekker, Inc., NY, pp. 1209–1234, 1984.

Thoen, C.O. and E.M. Himes. Pathogenesis of *Mycobacterium bovis* Infection. In *Progress in Veterinary Microbiology*. R. Pandy, S. Kargar, A.G. Basal, ed., Basal, Switzerland, in press, 1985.

Theon, C.O. and J. Haag na. Interspecies Transmission of Tuberculosis. In *Handbook on Epidemiology of Tuberculosis*. Sjogren, ed., International Union Against Tuberculosis, Paris, France, in press, 1985.

Thoen, C.O., C. Malstrom, and E.M. Himes. Use of Enzyme-linked immunosorbent assay for detecting mycobacterial antigens in tissue of *Mycobacterium bovis*-infected cattle. *Am. J. Vet. Res. 42*:1814–1815, 1981.

Timme, T.L. and P.J. Brennan. Induction of bacteriophage from members of the *Mycobacterium avium, Mycobacterium intracellulare, Mycobacterium scrofulaceum* serocomplesx. *J. Gen. Microbiol. 130*:2059–2066, 1984.

Tsang, A.Y. I. Drupa, M. Goldberg, J.K. McClatchy, and P.J. Brennan. Use of serology and thin layer chromatography for the assembly of an authenticated collection of serovars within the *Mycobacterium avium-Mycobacterium intracellulare-Mycobacterium scrofulaceum* complex *Int. J. Syst. Bacteriol. 33*:285–292, 1983.

Tsang, A.Y., V.L. Barr, J.K. McClatchy, M. Goldberg, I. Drupa, and P.J. Brennan. Antigenic relationship of the *Myobacteium fortiuitum-Mycobacterium chelonae* complex. *Int. J. Syst. Bacteriol. 34*:35–44, 1984.

Tweardy, D.J., B.Z. Schacter, and J.J. Ellner. Association of altered dynamics of monocyte surface expression in tuberculosis. *J. Infect. Dis. 149*:31–37, 1984.

Vandiviere, H.M., I.G. Melvin, R. Narian, W.D.M. Harris, and S.D. Chaparas. Profiles of skin test reactivity to antigens of various mycobacterial species in a human population and in experimental infections. *Tubercle 61*:245–257, 1980.

Vosika, G., J. Schmidtke, A. Goldman, R. Parker, E. Ribi, and G.R. Gray. Phase I Study of Intradermal Immunotherapy with Oil-Attached *Mycobacterium smegmatis* Cell Wall Skeleton and Trehalose Dimycolate. *Cancer Immunol. Immunother. 7*:221–224, 1980.

Vosika, G., and G.R. Gray. Phase I Study of Intravenous Mycobacterial Cell Wall Skeleton and Cell Wall Skeleton Combined with Trehalose Dimycolate. *Cancer Treatment Reports 67*:785–790, 1983.

Wallace, R.J., Jr., J.M. Swenson, V.A. Silcox, and R.C. Good. Disk diffusion testing with polymyxin and amikacin for differentiation of *Mycobacterium fortuitum* and *Mycobacterium chelonei. J. Clin. Microbiol. 16*:16:1003–1006, 1982.

Wallace, R.J., Jr., J.M. Swenson, V.A. Silcox, R.C. Good, J.A. Tschen, and M.S. Stone. Spectrum of disease due to rapidly growing mycobacteria. *Rev. Infect. Dis. 5*:657–679, 1983.

Watson, S.R., L.K. Auclair, and F.M. Collins. The effect of combined chemotherapy on suppressor T-cell activity in *M. simiae-infected mice. Immunol. 43*:459–465, 1981.

Watson, S.R., and F.M. Collins. Supressor T-cell production in heavily infected mice. *Immunol. 39*:1367–1373, 1980.

Watson, S.R., and F.M. Collins. The specificity of suppressor T-cells induced by *Mycobacterium avium* infections in mice. *Clin. Exp. Immunol. 43*:10–19, 1981.

Wayne, L.G., E.J. Krichevsky, L.L. Love, R. Johnson, and M.I. Krichevsky. Taxonomic probability matris for use with slowly growing mycobacteria. *Inst. J. Syst. Bacter. 30*: 528–538, 1980.

Wayne, L., et al. First report of the cooperative, open-ended study of slowly growing mycobacteria by the International Working Group on Mycobacterial Taxonomy. *Int. J. Syst. Bacter. 31*:1–20, 1981.

Wayne, L.G. Numerical taxonomy and cooperative studies: roles and limits. *Rev. Infect. Dis. 3*:822–828, 1981.

Wayne, L.G. Microbiology of the tubercle bacilli. *Amer. Rev. Resp. Dis. 125*(Supl.):31–41, 1982.

Wayne, L.G. and G.A. Diaz. Serological, taxonomic and kinetic studies of the T and M classes of mycobacterial catalase. *Int. J. Syst. Bacter. 32*:296–304, 1982.

Wayne, L.G., and K.-Y. Lin. Glyoxylate metabolism and adaptation of *Mycobacterium tuberculosis* to survival under anaerobic conditions. *Inf. and Immun. 37*:1042–1049, 1982.

Wayne, L.G. On the relationship of members of the tuberculosis complex to other species of mycobacterial. *Ind. J. Chest Dis. 24*:118–126, 1982.

Wayne., L.G. Microbiology of tuberculosis. In *Pulmonary Diseases and Disorders*. Fishman, A.P., ed., McGraw-Hill Book Co., NY, pp. 1255–1263, 1980.

Wayne, L.G., et al. Second report of the cooperative, open-ended study of slowly growing mbycobacteria by the International Working Group on Mycobacterial Taxonomy. *Int. J. Syst. Bacteriol. 33*:265–274, 1983.

Wayne, L.G. Las funciones del laboratorio clinico en el control de la tuberculosis: perspectivas y prioridades. *Revista de Laboratorio 75*:139–155, 1983.

Wayne, L.G. Classification of mycobacteria vs. classification of mycobacterial diseases. *Chest 86*:506–507, 1984.

Wayne, L.G., M.I. Krichevsky, D. Portyrata, and C.K. Jackson. Diagnostic probability matrix for identification of slowly growing mycobacteria in clinical laboratories. *J. Clin. Microbiol. 20*:722–729, 1984.

Wayne, L.G. Mycobacterial speciation. In *The Mycobacteria: A Sourcebook*. G.P. Kubica and L.G. Wayne, eds., Marcel Dekker, Inc., NY, pp. 25–65, 1984.

Wayne, L.G. Mycobacterial enzymes involved in generation and destruction of hydrogen peroxide. In *The Mycobacteria: a Sourcebook.* G.P. Kubica and L.G. Wayne, eds., Marcel Dekker, Inc., NY, pp. 595-601, 1984.

Weisman, L.S. and C.E. Ballou. Biosynthesis of the Mycobacterial Methylmannose Polysccharide. Identification of an c(1->4)-mannosyltransferase. *J. Biol. Chem. 259:* 3457-3463, 1984.

Weisman, L.S. and C.E. Ballou. Biosynthesis of the Mycobacterial Methylamannose Polysaccharide. Identification of an 3-0-methyltransferase. *J. Biol. chem. 259:*3464-3469, 1984.

Wendt, S.L., K.L. Gorge, B.C. Parker, H. Gruft, and J.O. Falkinham III. Epidemiology of infection by nontuberculous mycobacteria. III. Isolation of potentially pathogenic mycobacteria from aerosols. *Am. Rev. Resp. Dis. 122:*259-263, 1980.

Wolinsky, E. Significance of Medical Microbiology in the Care of Patients, V. Lorian, ed., Williams and Wilkins Company, Baltimore, MD, 1977. Chapter 10, Mycobacteria: Significance of Speciation and Sensitivity Tests, 2nd Edition, Chapter 8, 1982.

Wolinsky, E. Microbiolgy. Davis, Bulbecco. Eisen and Ginsberg, eds., Harper and Row, Inc., Hagerstown, 3rd ed., 1980: Chapter 37, *Mycobacteria.* ed.

Yabusaki, K K. and C.E. Ballou. Long Chain Fatty Acyl-CoA Thio-esterase from *Mycobacterium smegmatis.* In *Methods in Enzymology,* J. Lowenstein, ed., 242-246, 1980.

Yanagihara, D.L., V.L. Barr, C.V. Knisley, A.Y. Tsang, J.K. McClatchy, and P.J. Brennan. Enzyme-linked immunosorbent assay of glycolipid antigens: Application of mycobacteria. *J. Clin. Microbiol.* in press.

Yandell, P.M. and C. McCarthy. Isolation of deoxyribonucleic acid from *Mycobacterium avium* by rapid nitrogen decompression. *Infect. Imm. 27:*368-375, 1980.

Joint Scientific Conferences

TUBERCULOSIS PANEL

Date: October 6–8, 1980

Place: Gakushi-kaikan, Tokyo

Summary: The 15th Conference was held October 6–8, 1980 in Gakushi-kaikan, Tokyo. Eleven delegates participated from the U.S. Tuberculosis Panel. There were five sessions and a total of 20 papers were presented. The principal subjects presented to the Conference were 1) Chemistry and biological activities of lipids and glycolipids of mycobacteria, 2) Mechanism of granuloma formation, 3) Suppressor T-cell in the immunity in tuberculosis, 4) Strain differences in the development of delayed-type hypersensitivity, 5) Roles of macrophage in the immunity in tuberculosis, 6) Antitumor activities of the chemical constituents of mycobacteria.

Date: November 16–17, 1981

Place: Bethesda, Maryland

Summary: The 16th Conference was held November 16–17, 1981, at the National Institutes of Health in Bethesda, Maryland. There were four sessions at which a total of 25 papers were presented. The subjects of these sessions were: 1) The structure and function of mycobacteria, 2) Immunoregulation in tuberculosis and nontuberculous mycobacteria, 3) Biochemistry and genetic expression in mycobacteria, and 4) Various fractions and immune responses in mycobacteria. Stimulating discussions followed the presentation of each paper.

Date: July 19–21, 1982

Place: Takebashi-kaikan, Tokyo

Summary: The 17th Conference was held July 19–21, 1982 in Takebashi-kaikan, Tokyo. Eight delegates participated from the U.S. Tuberculosis Panel. Seven separate sessions evaluated the following relevant problems: 1) Biology of mycobacteria, 2) Defense mechanism against tuberculosis infection, 3) Host response against live BCG, 4) Biologically active constituents of mycobacteria, 5) *In vivo* and *in vitro* responses to PPD, 6) Immunological responses in human tuberculosis, 7) Granuloma and immune responses. A total of 24 papers were presented.

Date: August 3–5, 1983

Place: Bethesda, Maryland

Summary: The 18th Conference was held August 3–5, 1983, at the National Institutes of Health in Bethesda, Maryland. There were four sessions held on August 3–4, 1983, at which a total of 27 papers were presented. The subjects of these sessions were: 1) Cellular and immune mechanisms in mycobacteria, 2) Pathogenesis, models of immunity and phage of mycobacteria, 3) Immune regulation in mycobacteria and 4) Structure, function and antimicrobials of mycobacteria. There was a joint symposium between the Tuberculosis and Leprosy Panels on August 5, 1983. A total of six papers were presented on the immunology of mycobacteria. Active and constructive discussions followed the presentation of each paper.

Date: August 27–31, 1984

Place: Takebashi-kaikan, Tokyo

Summary: The 19th Conference was held August 27–31, 1984, under the cosponsorship with the Leprosy Panel in Takebashi-kaikan, Tokyo. On August 27 and 28, papers by members of the Leprosy Panel were presented. On August 29, a joint symposium was held, and on August 30 and 31, papers from the Tuberculosis Panel members were presented. The subject of the joint symposium was "Immunology in Tuberculosis and Leprosy". Six representative papers were presented from each of the Panels. The Conference on Tuberculosis was organized into four separate sessions including: the mechanism of suppression, macrophage and infection-protective immunity, biology and biologically active substances of mycobacteria, and mycobacterial lipids. A total of 23 papers were presented. Twelve delegates from the U.S. Tuberculosis Panel attended the conference. The joint meeting held with the Leprosy Panel resulted in objective and helpful discussions, regarded by everyone as productive.

VIRAL DISEASES PANEL

Drs. Konosuke Fukai and Thomas Yuill, Chairmen respectively of the Viral Diseases Panel, U.S.-Japan Cooperative Medical Science Program

Dr. Konosuke Fukai, Chairman (1979–84,
Member 1979–80)
Chairman, Board of Directors
Research Foundation for Microbial
Diseases of Osaka University
3-1, Yamadagaoka, Suita-shi
Osaka 565, Japan

Dr. Thomas M. Yuill, Chairman (1982– , Member 1976–80)
Associate Dean for Research and Graduate
Training
School of Veterinary Medicine
University of Wisconsin
2015 Linden Drive West
Madison, Wisconsin 53706

PANEL MEMBERS

Japan

Dr. Nobuo Hashimoto (1982–)
Professor
Faculty of Veterinary Medicine
Hokkaido University
N. 18, W. 9, Kita-ku
Sapporo 060, Japan

Dr. Akihiko Kawai (1984–)
Associate Professor
Institute for Virus Research
Kyoto University
Shugoin Kawaramachi, Sakyo-ku
Kyoto 606, Japan

Dr. Tasuke Konno (1980–)
Professor
Department of Pediatrics
Research Institute for Tuberculosis
and Cancer
Tohoku University
4-1, Seiryo-machi
Sendai-shi 980, Japan

United States

Dr. Albert Z. Kapikian (1978–)
Assistant Chief
Laboratory of Infectious Diseases
National Institute of Allergy and
Infectious Diseases
National Institutes of Health
Building 7, Room 103
Bethesda, Maryland 20205

Dr. Neal Nathanson (1979–)
Professor and Chairman
Department of Microbiology
School of Medicine
University of Pennsylvania
Philadelphia, Pennsylvania 19174

Dr. Phillip K. Russell (1983–)
Commander
Fitzsimons Army Medical Center
Aurora, Colorado 80045

Dr. Akira Oya (1975–)
Director
Department of Virology
and Rickettsiology
National Institute of Health
2-10-35, Kamiosaki, Shinagawa-ku
Tokyo 141, Japan

Former Members

Dr. Reisaku Kono, Chairman
(1977–79, Member 1971–77)
Deceased (1985)
Professor
Department of Bacteriology
Saitama Medical School
Moroyama-Hongo, Moroyama-cho,
Iruma-gun
Saitama Prefecture 350-04, Japan

Dr. Seiichi Matsumoto (1976-1983)
Deceased (1985)
Professor
Institute for Virus Research
Kyoto University
Shugoin-Kawaramachi, Sakyo-ku
Kyoto 606, Japan

Former Members

Dr. John J. Holland (1979–83)
Professor
Department of Biology
University of California at San Diego
San Diego, California 92037

Dr. William F. Scherer (Chairman 1978–80,
Member 1974-1978) Deceased (1982)
Professor and Chairman
Department of Microbiology
Cornell University Medical College
New York, New York 10021

Japanese Viral Diseases Panel

BROAD GOALS

Introduction

The remarkable success in suppression of bacterial infections by the introduction of antibiotics has focused worldwide attention on viral diseases as important human health problems. In developed countries, control of many viral diseases has been successful through public health measures. In developing countries, however, the situation is more serious because of many restrictions on public health resources. This is also true in Asian countries where several hundred millions of people are menaced by viral epidemics.

Transition of priority fields

The Japan-U.S. Joint Panel on Viral Diseases selected four important diseases for study when the joint panel was formed. These were: smallpox, respiratory diseases, diseases caused by arboviruses, and rabies. These included epidemiology and ecology; molecular biology; pathogenesis; preventive measures including environmental control and modification of vector competence; and the development of new vaccines. Japanese Panel Members planned cooperative, domestic studies on these diseases important to Japan as well as to Asia.

At the Ninth Viral Disease Panel meeting in 1975, it was mutually agreed that research would be focused on rabies, dengue, and other arboviruses. However, in 1978 the guidelines of the Joint Viral Diseases Panels were expanded by the Joint Committee to include viral gastroenteritis, one of the most common infections of humans in developed and developing countries.

Present scope of the Program

At present, the scope of the Program of the Joint Panel on Viral Diseases includes important and prevalent diseases common to the United States, Japan, and Asia. These include dengue, viral gastroenteritis, and rabies, all of which are important causes of human morbidity and mortality. The Program is cohesive with common research guidelines between the Japanese and U.S. panels. These guidelines include epidemiology, pathogenesis, molecular biology, diagnosis, prevention, and control measures for rabies, arbovirus infections, and viral gastroenteritis.

PROGRESS IN PRIORITY AREAS IN 1980-84

Rabies

Pathogenesis of rabies virus infection

Remarkable progress was achieved in the study of the mechanism of viral dissemination in the brain. Using the electron microscope, an investigator examined brain tissue of patients who recently died of rabies in China to determine the site of virus maturation. A number of viral budding processes were found at sites which seemed to be neuronal synapses. This finding may explain the fundamental process of viral spreading in the brain.

Several studies elaborated on high-egg-passaged (HEP-Flury) rabies virus, an avirulent strain, and on its neurovirulent revertants. It was reported that HEP-Flury rabies virus which was passed serially through chick embryo fibroblast cells failed to kill even nude mice and cyclophosphamide-treated mice by intracerebral inoculation. Partial virulence for adult mice was regained after three passages in mouse neuroblastoma cells (NA strain of C1300 cell). Even after 20 passages through NA cell cultures, the virus did not regain its virulence to the same degree as revertants obtained by suckling mouse brain passages. A comparison was made of the avirulent HEP strain with its neurovirulent revertants. Virulent rabies virus replicated very slowly and induced only small amounts of interferon in the early phase of infection. On the other hand, rapid replication of avirulent virus and concomitant production of large amounts of interferon were observed but restricted to the early phase of infection. These data suggest that the fate of infection of each type of rabies virus might be related to events occurring in the early phase of infection.

Post-exposure vaccination and immunopathology

Other investigators continued the study of post-exposure vaccination. Mice were intracerebrally inoculated with a modified street virus from persistently infected mouse neuroblastoma cells. Two hours later they were treated with rabies vaccine that had been prepared in chick embryo cell culture. All vaccinated mice survived, while 10 to 30% of unvaccinated mice showed typical symptoms of illness. Administration of cyclophosphamide 1 day before and 1 day after vaccination resulted in the death of all mice.

The role of cell-mediated immunity in the treatment by post-exposure vaccination was studied. It was noted that co-cultivation of infected mouse neuroblastoma cells with spleen cells prepared from immunized mice suppressed the formation of fluorescent antibody (FA) foci. This effect was attributed to T cell function. Neutralizing antibody, interferon, and lymphotoxin were not detected in the co-cultivation medium.

It is well known that post-exposure vaccination sometimes causes two undesirable effects: allergic encephalitis caused by contaminating cellular basic proteins in the crude vaccine preparations, and the so called "early death." The latter refers to some rabies vaccinated persons exposed to street rabies virus who develop the disease with a shorter latent period and die more quickly than those not vaccinated. Investigators tried to analyze this "early death" phenomenon by establishing an animal model system which mimicked the human phenomenon.

One group employed corneal inoculation of animals with street rabies virus which was followed by intraperitoneal inoculation of inactivated vaccine. They noted that the incubation period for developing clinical illness was reduced. Another group observed the "early death" phenomenon in mice which had received an intracranial inoculation with rabies virus, CVS strain, followed by intraperitoneal vaccination. An increase of neutralizing antibody levels in serum was found 5 days after infection. However, passive transfer of immune serum or spleen cells obtained from immunized mice to infected mice did not result in early deaths. Furthermore, the group recently observed the phenomenon in monkeys and guinea pigs, suggesting that "early death" is a common phenomenon in many animals, as well as in humans. The establishment of animal models, hopefully, will lead to an elucidation of the mechanism of "early death."

Development of a new vaccine

A continuous supply of effective, low-cost, and safe vaccine is needed in developing countries where rabies is prevalent. It is very difficult for these countries to produce their own tissue culture vaccines. One investigator and his colleagues tried to develop an improved rabies vaccine applicable in developing countries.

Virus-cell interactions in cell culture

Neuroblastoma cell lines exhibit various neuron-like properties *in vitro* and have served as the model host for *in vitro* studies of the neurotropic rabies virus infection. Studies of a rabies virus infection in a human neuroblastoma cell line (SYM-I strain) showed that a HEP rabies virus infected SYM-I cell produced considerable amounts of interferon. Fewer progeny virions were produced within 3 days of infection. At the second passage, the virus yield dropped to 1/1000 of that of the first passage. Persistent infection of HEP virus was easily initiated in SYM-I cells, in which interferon and defective interfering (DI) particles were produced continuously.

Many rhabdomyosarcoma cell lines of mouse origin have been established. These cell lines resisted the cytopathic effect (CPE) caused by rabies CVS strain and became persistently infected. Further studies are expected in these areas which may offer clues to understanding viral behavior during the long latent period.

Molecular biology of rabies virus

Recently it was found that the shift-up of incubation temperature of HEP virus-infected BHK cell cultures induced an accelerated CPE even though replication of virus stopped at the higher temperature. By raising the incubation temperature, diverse molecular changes were induced in replication, such as the termination of genomic RNA synthesis and up to a tenfold increase of viral mRNA synthesis. This was not followed by an increase of viral protein synthesis. This suggested that viral regulation of RNA synthesis may be temperature sensitive, but the regulatory function is not associated with the temperature sensitivity of accelerated CPE.

The rabies virion is composed of at least five species of viral proteins (L, G, N, M1, and M2 proteins) and usually has some additional minor protein components, such as acting molecules of cellular origin and p40 proteins. Immunological and biochemical studies indicated that p40 was a modified M1 protein. Both p40 and M1 proteins are phosphoproteins, but p40 was much more phosphorylated than M1 protein. M1 protein was found abundant in infected cells, but accounted for only 6% of mature virions. On the other hand, p40 protein was only detected in mature virions (3%–4%). It was suggested that p40 protein in the mature virion was produced by re-phosphorylation of M1 protein at or during the process of viral assembly to maturation.

Developments in laboratory techniques

The hemagglutination-inhibition (HI) test is one of the methods used for rapid antibody assay for study of many types of viruses. Rabies virus agglutinates goose erythrocytes at a low pH and when a nonspecific serum inhibitor (lipoprotein) is removed by kaolin. This technique, however, has not been used in rabies research because of the low sensitivity of the test in detecting viral proteins and difficulties in completely eliminating the nonspecific inhibitor(s). There were attempts to overcome these faults associated with the rabies virus HI test: treatment of test sera with colloidal siliasic acid (Aerosil) and acetone was adequate to remove the nonspecific inhibitory; and treating goose erythrocytes with a low concentration (0.005%) of Bromeline significantly increased their agglutinability of rabies virions. Combining these procedures, low titers of antirabies antibodies were detected efficiently. The HI antibody titers obtained with these improved procedures became 3 to 6 times higher than those obtained with the current procedures. Low levels (under 1:20) of antibodies in vaccinated human sera could efficiently be measured with higher sensitivity. This improved HI test for rabies virus is now expected to be used widely in rabies research. With this improved HI test, antigenic differences among some strains of rabies virus could be detected in parallel with cross-neutralization tests. The importance of the selection of HA antigen in the HI test for seroepidemiological studies is suggested.

A sensitive bioassay method was developed for detecting small amounts of DI particles. Increasing the incubation temperature to 40C after an initial period at 36C, initiated a rapid CPE and a cessation of viral replication. When this procedure was applied to a mixed infection of rabies virus and DI virions, clusters of surviving cells stood out against a background of lysed cells. With this finding, an interference focus-forming assay of rabies virus DI particles was devised. Under optimal conditions this procedure was sufficiently sensitive to measure as little as 20 IFU/ml of DI particles with an efficiency almost equal to the plaque assay.

Arboviruses including dengue viruses

A variety of research was conducted, with arboviruses in the fields of epidemiology, immunology and molecular virology under the present program.

An analysis was made of the biological function of two glycoproteins, E1 and E2, located on the surface of western equine encephalitis (WEE) virus, an alpha virus. Separation of two glycoproteins by discontinuous SDS-gel electrophoresis and SDS-hydroxylapatite chromatography were found to cause denaturation of those proteins; their biological activities were eventually lost. The following procedures were initiated to improve separation: 1) isoelectric focusing in a urea gradient, 2) ion exchange chromatography following Triton X-100 treatment at different pH and ionic strengths, and 3) glass wool chromatography on the basis of their differential binding to glass wool in the presence of NP40. Glass wool column chromatography was successful, and an attempt was made to identify proteins which are responsible for adsorption to cells. E1 protein hemolyses red blood cells.

Chikungunya (CHIK) virus is found widely in Africa and Asia as a pathogenic alphavirus for man. Some characteristics of its structural proteins have not been clarified. Studies were performed comparing CHIK protein components with those of well analyzed alphaviruses with the following results: 1) the envelope proteins E1, E2, and capsid protein were released in the medium by cleavage of PE2; 2) E2 was produced by cleavage of PE2; and 3) separation of E1 and E2 was rather difficult compared to Semliki forest virus. It was suggested that properties of CHIK virus structural proteins were more similar to WEE virus than to Semliki virus. However, serologically CHIK is grouped in the Semliki virus subgroup.

An analysis was performed of the antigenic structure of the envelope V3 protein of Japanese encephalitis (JE) virus using monoclonal antibodies. Eleven (11) monoclonal antibodies were assigned to 4 groups; flavivirus-cross-reactive HI and non-neutralizing antibody (group 2), low HI and neutralizing antibody (group 4 and 5). The results of their competitive binding assay showed the existence of at least five distinct antigenic determinants of V3 protein. HA sites could be separated from neutralization sites. Two HA sites were identified, one, flavivirus-cross-reactive and the other, subgroup specific.

Attempts were made to determine the structure of the JE virus genome by using molecular cloning methods. Temperate JE virus RNA was used to prepare JE virus cDNA. cDNAs were inserted into the Pst-I site of pUC-12 DNA for cloning. More than 20 clones were obtained which hybridized with JE virus probes. A physical map of each clone was examined and clones were classified into four groups. It was estimated that the clones cover approximately 7 kilo-bases which occupied more than half of the JE virus genome. Studies are in progress to obtain cDNA of full length and to sequence all of the JE genome.

Participation of cellular immunity in JE virus infection

The role of cellular immunity in experimental mice infected with JE virus was investigated. C3H/He mice were thymectomized after ^{60}Co irradiation. Treated mice received thymus cells or bone marrow cells separately or both cells simultaneously and were inoculated with JE virus intravenously. Most of the mice receiving both types of cell survived. However, all mice given only one type of cell died. Remarkable infiltration of mononuclear cells in the brains and meninges was observed in mice receiving both cell types. Also, mice receiving both cell types produced IgM and IgG antibodies against JE virus, but mice receiving only B cells produced only IgM antibody. Cells collected from brains with mononuclear cell infiltration contained cytotoxic T cells and showed viral clearance activity in JE virus-infected cultured cells. These activities were higher in brain cells than in spleen cells. They found that ^{60}Co irradiated mice inoculated intracerebrally with JE virus could be protected from death by transfer with immune spleen cells, but signs of the disease appeared earlier in the immunized mice than the unimmunized mice. They suggested that the infiltrating cells were essential for viral clearance in the brain, although they accelerated some early processes of disease.

Characteristics of cellular immunity in JE virus infection were investigated in mice. Cytotoxic cells were observed from day 5 and reached a peak 77 days after infection in mouse spleens. Cytotoxic activity was clearly demonstrated in T cells but little in B cells. Interestingly, protective activity was noted with B cells but was scarce with T cells. Protection was not successful when experimentally infected mice were treated as late as 4 days after infection either by antiserum or by immune spleen cells. Apparent protection, however, was shown when both treatments were combined. Spleen cells from mice immunized with inactivated virus showed protective activity and no cytotoxic activity. Accordingly, it was suggested that immune B cells, with the cooperation of specific antibody, play an important role in the cellular immunity in JE virus infection.

Application of ELISA to arbovirus serology

The ELISA test was found more sensitive than the HI test for detecting antibody to JE virus in pig serum. ELISA was used for the diagnosis of human JE. Sera from 460 suspected JE cases and sera from healthy residents in non-endemic areas of JE were examined. JE cases were confirmed when a 4-fold titer increase was obtained with paired sera. "Probable" JE was diagnosed with a single sample of serum when a titer as high as 10,000 or more was obtained with whole immunoglobulin or more than 100 with IgM. JE negativity was confirmed when the titer with whole immunoglobulin showed less than 800, even in the convalescent stage.

Antibody response was analyzed by ELISA using guinea pigs after infections with JE and West Nile viruses. The infections were separated by an appropriate time interval. It was found that antibody titers after the second infecting virus were consistently higher against the initial infecting virus, regardless of order of the infecting viruses. It was hypothesized that the theory of "original antigenic sin" could be applied in the case of flavivirus infection as well as with influenza viruses.

The biotin-avidin ELISA system was successfully used to measure JE antibody. It was emphasized that the biotin-avidin ELISA system showed less nonspecific reaction than ordinary ELISA and was useful in measuring JE antibodies in sera of various animal species. ELISA was applied for diagnosing JE and dengue cases in Japan and in Thailand with reference to antibody titers from normal individuals. The IgG-ELISA in JE cases in Thailand was equally as high to both JE and dengue antigens. It was confirmed that IgM capture ELISA was applied usefully to differentiate dengue and JE cases in Thailand.

An outbreak of a febrile exanthematous disease occurred among race horses in a race horse training center in Tochigi Prefecture in eastern Japan in the fall of 1978. Virus was isolated from blood samples of sick horses, and the isolated virus, almost identical to Getah virus, was identified as the causative agent. It was the first report of Getah virus illness in horses. The main signs of disease in the sick horses were light fever, generalized exanthem, and cold edema of the joints. Illness lasted for 2 to 5 days. Inapparent infection of horses occurred frequently. Experimental infection of horses with the isolate showed the incubation period to be approximately 1 week. Viremia was observed for 5 days after the onset of the disease. Sick horses showed neutralizing, complement-fixing, and hemagglutination-inhibiting antibodies in convalescent sera. C6/36 mosquito cells were used successfully for isolating the arbovirus.

The effectiveness of the C6/36 mosquito cell line was compared with suckling mice for the isolation of arboviruses from mosquitoes collected in nature. The mosquito suspensions were centrifuged and the supernatants were inoculated into suckling mouse brains. The same suspensions were inoculated onto a monolayer of C6/36 cells after Millipore filtration. Seventeen strains of JE and five strains of Getah viruses were isolated by C6/36 cells, whereas eight strains of JE and two strains of Getah were isolated by suckling mouse brain inoculation. These results suggested that C6/36 cells were more sensitive for virus isolation than suckling mouse brains.

C6/36 cells were used for dengue virus isolation from patients with dengue hemorrhagic fever (DHF) in Bangkok. Eight CP positive strains were isolated and identified as dengue type 2 virus by IAHA. After freezing and thawing the supernatants of the CP negative cultures, nine dengue viruses were isolated and identified by the application of IAHA. The results demonstrated quite clearly the applicability of C6/36 cells for virus isolation, and the IAHA for identification of dengue viruses.

A research worker was accidentally infected in the laboratory with dengue virus isolated from a patient in Thailand in 1978, preliminarily identified as type 4 dengue virus. Two strains of virus were reisolated from this patient's serum on the 1st day of illness by inoculating serum onto C6/36 cells or into suckling mouse brains. The convalescent serum showed a neutralizing antibody titer of 4,000 against the C6/36 reisolate. Surprisingly, the same serum showed a neutralizing antibody titer of onl[illegible]0 against the mouse brain reisolate. When the mouse brai[illegible]as was passed through C6/36 cells, the NT titer was much [illegible]oved. When the C6/36 cell reisolate was passed though suckling mouse brain, the NT was decreased. Such a distinct host dependent change of the NT antibody reactivity of dengue virus called attention to factors that could affect the serological diagnosis of dengue.

Ecological studies of arboviruses

The magnitude of human JE epidemics and weather factors were analyzed for Nagasaki Prefecture over the period of 1950 to 1979. The size of the epidemic demonstrated a negative correlation with th[illegible] summer precipitation and a positive correlation with the [illegible]ummer temperature. Larger epidemics occurred in the years with light precipitation and high temperatures. High temperatures may accelerate the multiplication of JE virus in the vector mosquito. Light precipitation and high temperatures may bring about an increase in the vector population since the survival rate of immature mosquitoes is larger through shortened developmental periods. Also, light precipitation may minimize the loss of immature mosquitoes by water currents.

An ecological study of Getah virus was conducted in Japan. An investigator examined 27,000 mosquitoes of 6 species for the natural presence of Getah virus. Among a total of 18 Getah virus isolates, 15 were from *A. vexans*, and 3 from *C. tritaeniorhynchus*. Virus isolation was successful from September to October when *A. vexans* peaked in nature. Getah virus was found widely disseminated among swine. It is assumed that *A. vexans* is the main vector and that swine play a role as the main amplifier of Getah virus in nature.

More than 20 strains of Getah virus isolated from mosquitoes, swine, and horses in Japan during 1956 to 1981, and one prototype strain from Malaysia, were analyzed by investigators using the RNA oligonucleotide finger-printing method. It was reported that the prototype strain was quite different from the Japanese isolates. Although most Japanese isolates shared many large oligonucleotides in common, their patterns were not identical even among isolates from the same place in the same year. The results indicate a high mutability of Getah virus.

A field study of abovirus was carried out in Chiang Mai of northern Thailand, in July and August 1982. One strain of JE, 11 strains of dengue, and 2 unidentified flaviviruses were isolated. Dengue virus was isolated from one case of encephalitis and one case of aseptic meningitis. A seroepidemiological survey revealed that the infection with JE virus was not uniformly scattered throughout the Chiang Mai Valley. An HI antibody survey of vertebrate sera showed high positive rates to JE virus in swine, horses, mules, and sheep; lower rates in monkeys, ducks, and sparrows; and no antibody in chickens and lizards.

A serological survey of Apoi virus was carried out in Hokkaido. Sera from a variety of animals, including humans from different districts of Hokkaido, were tested for Apoi antibody by the plaque neutralization test or by ELISA. Three of 659 humans, 116 of 1,384 bovines, 5 of 520 horses, 2 of 296 pigs, 1 of 4 brown bears, and 1 of 123 wild rodent sera were positive. IgM antibody was detected in young calves.

Viral Gastroenteritis

Rotavirus infections

Recently, a major advance was made in *in vitro* cultivation of human rotaviruses, which allowed almost routine isolation of human rotaviruses. The MA104 cell line derived from fetal rhesus monkey kidneys and primary cultures of either African green or cynomolgus monkey kidney cells were sensitive to human rotaviruses following trypsin treatment of the virus prior to and during cultivation in roller tube cultures. With these procedures, a number of rotavirus strains were isolated in Japan and other countries.

The successful propagation of human rotavirus in cell culture has facilitated their classification according to neutralization specificity. Among rotavirus strains isolated in Japan, four different serotypes have been distinguished in collaboration with colleagues in the United States: serotype 1 (K8, KU), serotype 4 (Hosokawa, Hochi). The major protein responsible for neutralization specificity is coded for by the eighth or ninth RNP segment, the product of which is an outer capsid glycoprotein (Vp7) with a molecular weight of 34-38 kilo-dalton (K-D). Antisera against bovine rotavirus Vp7 have, in addition to neutralization, hemagglutination inhibition (HI) and complement-fixation (CF) activities. Rotavirus strains belonging to serotypes 1, 3, and 4 have subgroup 2 specificity, whereas strains belonging to serotype 2 have subgroup 1 specificity. Subgroup specificity is determined by the major inner capsid protein (Vp6) with a molecular weight of 41 K-D, the product of RNP segment 6 which also has common group antigens.

It was shown that human rotaviruses propagated in the absence of trypsin have the ability to agglutinate day-old chicken erythrocytes. Trypsin treatment decreases the hemagglutination titer of human rotavirus and, conversely, increases their infectivity.

Monoclonal antibodies against the hemagglutinin of rotaviruses (including human, simian [SA-11] and bovine [NCDV] rotaviruses) proved to have neutralizing activity against the homologous virus strain, as well as hemagglutination-inhibition activity. In SA-11 and Vp88, the outer capsid protein and the product of the fourth gene segment is immunoprecipitated with monoclonal antibody directed at HA, and cleaved by trypsin to produce Vp60 and Vp28.

One group proposed a potential live candidate vaccine by preparing a cold-adapted strain of human rotavirus (IGV-80-3) serially passaged in primary monkey cells at temperatures shifted down stepwise. The 25 C-adapted rotavirus showed no pathogenicity in pigs.

It was found that morphogenesis of rotavirus occurs at the membrane of the rough endoplasmic reticulum, besides single-shelled particles synthesized in the viroplasma. Double-shelled virions arising at the endoplasmic reticulum membrane enter the cisternae through an exocytosis-like process. The "enveloped" particles, releasing single-shelled particles during cytolysis, appear to be the result of inefficient virus maturation at the last stage of outer capsid formation.

Polyacrylamide gel electrophoresis of viral RNA proteins allows a classification of rotavirus genotypes. Human rotaviruses, however, show extensive diversity in their genomic patterns (electropherotypes). Epidemiological studies using this procedure showed that a single but different dominant rotavirus electropherotype appeared to initiate each of the outbreaks, each remained important for the first 2-3 months.

When IgA antibodies against rotavirus in feces are determined, using a sensitive ELISA assay technique, the fecal IgA antibodies appear 1 week after the onset of the illness and decrease in titer 3 to 5 weeks later. In reinfection the IgA antibody responses are faster in appearance and higher in titer than those in primary infection.

Diarrheal viruses other than rotavirus

In general, 5%–10% of infants and young children with acute diarrhea shed fastidious adenoviruses when examined by electron microscopy. The majority of such fastidious enteric adenoviruses (Ead) represent new serotypes, types 40 and 41. An outbreak of gastroenteritis using hybridization techniques and immunoelectronmicroscopy in Japan was confirmed to be caused by Ead40.

Human calicivirus was recently to be cultivable in dolphin cells and shown to contain single-stranded RNA as genomic material. Community and nosocomial outbreaks of gastroenteritis associated with human calicivirus were reported in Japan, and in other countries. For detection of human calicivirus in stools, a microtiter solid phase radioimmunoassay was developed.

Astroviruses have been associated with diarrhea in several animals. An outbreak of astro-associated gastroenteritis occurred among children in a kindergarten in Japan.

Otofuke agent, 35–40 nm in diameter, containing a single-stranded DNA genome, was detected in patients with epidemic gastroenteritis in Japan. However, the etiological significance of this virus isolation remains to be established.

Hemorrhagic Fever With Renal Syndrome

Nomenclature

Hemorrhagic fever with renal syndrome (HFRS) was described in various countries under different names, e.g. epidemic hemorrhagic fever (EHF) in China and Japan, Korean hemorrhagic fever (KHF) in Korea, Far Eastern nephrosonephritis in the USSR, or nephropathia epidemica (NE) in Scandinavian countries. The Working Group on Hemorrhagic Fever convened in Tokyo, by the WHO Western Pacific Regional Office, recommended the use of the term "hemorrhagic fever with renal syndrome" in February 1982.

Present status

In Japan, about 120 cases of HFRS occurred between 1965 and 1975, mainly in Osaka City. Nearly 150 HFRS patients related to the handling of experimental rats have been registered in Japan from 30 institutions since 1970.

Since the isolation of the causative virus of KHF, Hantaan virus, in Korea in 1976, a serological survey of human and rodent sera became possible. The etiological relatedness of the various forms of HFRS in the Eurasian continent was established. Moreover, HFRS antibody in human sera was found in areas where no clinical HFRS has been recognized, e.g. in Bolivia, the Central African Republic, Gabon, Kenya, Egypt, Iran, India, Thailand, Philippines, Fiji, Hong Kong, Malaysia, Canada, and the United States.

Antibody prevalence among wild rodents was found worldwide: *Rattus Norvegicus* in Japan (Tokyo, Yokohama, Kobe, Hokkaido, Shimizu), *R. norvegicus* and *Microtus pennsylvanicus* in the U.S.A. (Houston, New Orleans, Philadelphia, Baltimore), *Microtus californicus* in California, and *Clethrionomys rutilis* in Alaska, etc.

Three groups of scientists are actively engaged in HFRS research in Japan. Several strains of Hantaan-related virus were isolated from rats in Japan in association with HFRS outbreaks, as well as handling of rats for experimental use. The prototype virus, Hantaan 76–118 strain, was isolated by means of *Apodemus* mouse inoculation. Recent strains, however, were more efficiently isolated by direct inoculation into cell cultures, especially onto VERO-E6 cells. There appear to be several types of Hantaan-related viruses.

From rats in Tokyo and in Hokkaido, HFRS virus antigen and other Hantaan-related viruses were detected in their organs. However, no clinical HFRS has been detected in either city. One strain, isolated from an experimental tumor-bearing rat in Osaka, was unique since it showed more affinity for rats than mice.

Diagnostic procedures employed successfully for HFRS antigen or antibody detection are: 1) indirect fluorescent antibody staining (IFA); 2) neutralization in cell culture (NT); 3) ELISA; 4) immune adherence hemagglutination (IAHA); 5) reverse passive hemagglutination inhibition (RPHI); and 6) complement-fixation tests (CF). IFA, ELISA, and RPHI are group-specific. IAHA, NT, and CF are type-specific and support epidemiological analyses on the basis of antigenic differentiation of the causative viruses.

Efforts to establish an animal model for human HFRS were not successful. Intracerebral inoculation into the suckling mouse or rat causes infections of varying degrees depending upon the pathogenicity of the virus strain used.

Mode of transmission among *Apodemus* populations was shown to occur by inhalation of dust contaminated with virus excreted in urine.

Taxonomic position of HFRS agents

HFRS agents were classified as members of the Bunyaviridae according to virion morphology and segmentedness of the RNA genome. All agents appear to be related to one another but differ to some extent from other groups of viruses in the genera Bunyaviridae. A new genus, "Hantavirus", among the Bunyaviridae was proposed for the cluster of HFRS agents.

Hemorrhagic fever with renal syndrome (HFRS) virus occurs in the lungs of Norwegian rat (Rattus norvegicus).

SUMMARY OF PANEL ACTIVITIES IN PAST 20 YEARS

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

FUTURE GOALS

[illegible]

[illegible]

Rabies

The molecular analysis of rabies virus must be explored further. Host-virus relationships, including pathogenesis and immunological aspects of rabies virus infection, especially in the central nervous system, needs much more investigation. The cause of the "early death" phenomenon is another problem to be clarified.

Arboviruses Including Dengue

Although Japanese encephalitis is uncommon in Japan, its incidence is increasing in other parts of Asia. Knowledge of the pathogenesis of Japanese encephalitis, especially in humans, is not adequate. Further understanding of the epidemiology of this disease, including the mechanism of overwintering of the virus in Temperate and Subtemperate Zones, the antigenic diversity of virus strains, and the development of adequate preventive measures remain major goals.

Dengue hemorrhagic fever with and without shock syndrome, remains of major importance in Asia. Before prevention and control can be implemented, a concentrated effort is necessary to develop better understanding of pathogenesis. Vaccine development is a program objective and work continues.

It should be emphasized that HFRS must be explored extensively as a global health problem.

Viral Gastroenteritis

The prevalence of human viral gastroenteritis, in infants and other age groups, is a greater problem in the Third World than in developing countries. Pioneer global studies are required to determine the various types of causative agents responsible for morbidity and mortality. Adequate channels between developing and developed countries must be established as soon as possible to help resolve this important health problem. Possibilities of developing effective preventive and control measures should be investigated thoroughly, drawing on knowledge gained from such actively developing fields as immunobiology and molecular genetics.

Development of more effective vaccines against rabies, Japanese encephalitis, dengue, and viral gastroenteritis, available for use, even in developing countries, is strongly encouraged and supported through research projects of the Joint Panel activities. Great contributions of growing biotechnology are anticipated in this field.

Research on pathogenesis of viral diseases and the mechanism of vaccine efficacy are essential companion fields of investigations in light of new immunological knowledge.

Field trials of new vaccines, demonstrated to be efficacious and safe against these viral diseases should be promoted as joint projects. It is necessary to establish appropriate understanding and consent of the specific national government, WHO, and other related organizations in order to implement these projects.

U.S. Viral Diseases Panel

BROAD GOALS

Ten years ago, the Ninth Viral Diseases Panel identified dengue and other arboviruses, rabies, and viral gastroenteritis as priority research areas. These categories were defined and addressed broadly in subsequent research studies and panel discussions. Arboviruses have included Korean hemorrhagic fever with renal syndrome. To obtain a better understanding of rabies, other rhabdoviruses were used as model systems for determining infection and viral persistence. The focus of viral gastroenteritis was primarily on rotaviruses, although other etiologic viral agents were investigated and discussed. In order to keep informed of current virologic problems, special relevant topics were selected for discussion at joint panel meetings.

The panels have not diminished their interest and concern regarding viral diseases which are significant causes of human morbidity and mortality around the world. The relative importance presented by these explosive illnesses and the capacity to make significant progress by U.S. and Japanese scientists are the principal reasons for maintaining the priority objectives of the joint panels. Panel meetings served to continually assess and re-evaluate the health importance, distribution, and epidemiology of these diseases and provided timely review of research in the U.S. and Japan. These reviews, and the professional contacts made through joint panel meetings fostered collaboration and cooperation between U.S. and Japanese scientists through discussion and interchange of techniques, reagents, and personnel. Consequently, scientific progress has been notable in all of the selected areas, especially in viral gastroenteritis and hemorrhagic fever with renal syndrome. Cooperative joint efforts resulted in the development of crucial new techniques, characterization of the etiologic agents involved, and inroads to understanding their function at the cellular level. Much remains to be accomplished before an adequate understanding of viral interaction, pathogenetic mechanisms, and epidemiologic clues provide the necessary leads to effective control.

DENGUE AND OTHER ARBOVIRUSES

Although the study for arbovirus gene function has progressed rapidly for alpha and bunyaviruses, the flaviviruses remain difficult to investigate which impedes the development of vaccines and application of new vaccine technology.

A fundamental understanding of alpha and flavivirus-vector relationships in nature is needed to match recent progress made with bunyaviruses and their vectors. This knowledge will help explain interepidemic persistence. More complete evidence for transovarial or venereal transmission under experimental and natural conditions is needed. Clarification of the impact of environmental factors such as temperature on virus replication and spread in vectors merits further investigation. Experiments are needed to show the nature of virus-cell interactions in vectors in order to understand tissue tropism/barriers as mechanisms of vector competence. There is need for further work on the genetics of vector competence.

The pathogenic mechanism of dengue hemorrhagic fever shock syndrome remains controversial. There are two working hypotheses and modern technology is reaching the state which should permit their testing. Studies of the virus-tissue interactions at the cellular level in vertebrates will provide useful insights to help describe and explain basic mechanisms of viral pathogenesis.

VIRAL GASTROENTERITIS

The rapid recent advances in elucidation of rotavirus structure and cellular function provide a basis for vaccine development, production, and testing. These developments create the need for complete understanding of viral pathogenesis and intestinal immunity. Appropriate studies are handicapped by lack of an appropriate and inexpensive vertebrate model system to test human rota and other viruses that cause gastroenteritis. A more complete understanding of the worldwide distribution of rota and other gastrointestinal viruses is urgently needed to assess the geographic distribution and relative importance of these agents as causes of human and animal diseases. Recent advances in diagnostic technology now make it possible to make these evaluations for rotaviruses. The importance of illnesses caused by non-rota virus agents will remain difficult to assess until diagnostic technology becomes adequate and relatively inexpensive. These epidemiological assessments make it necessary to establish priorities for various control measures which include vaccine development, based on reliable cost/benefit analyses.

RABIES

There is continued need for effective, safe, inexpensive, and available rabies vaccines for human, domestic animal, and wildlife administration. Advances in the understanding of rabies virus structure and its genetic control must be made if rabies is to be controlled in the known endemic areas. A more complete understanding of rabies virus pathogenesis will help improve approaches to prophylaxis, treatment, and control. Better diagnostic techniques and reporting methods must be developed before national, regional and international rabies control programs which employ modern vaccine technology, can be successfully and economically conducted.

SUMMARY OF THE FIRST FIVE YEARS

Research was oriented toward specific viral diseases of Asia including smallpox, rabies, and the arthropod-borne viruses.

The gel-precipitation test for diagnosis of smallpox is specific, rapid, and is 92% as sensitive as the more time-consuming technique of virus isolation. Analysis of subunit antigens of variola virus showed the presence of three specific hemagglutination antigens, and a high molecular weight precipitin which reacts with a neutralizing antibody.

The nucleocapsid of rabies virus is composed of RNA plus a single protein, which fixes complement. The coat glycoprotein is the hemagglutinating component. A newly developed hemagglutination test is comparable in its accuracy to the serum-neutralization test. In pathogenetic studies in foxes, it was shown that rabies virus matures on plasma membranes in the salivary glands. In nerve cells, intracytoplasmic maturation dominates. A search for clarifying over-wintering mechanisms of Japanese encephalitis virus in Korea and for attenuated vaccine strains was unsuccessful. Eleven mutants of *Culex tritaeniorhynchus*, the principle vector of Japanese encephalitis, were developed and show promise for control by genetic manipulation of mosquitoes.

Dengue type-2 virus was shown to contain three proteins, a coat glycoprotein of 59,000 MW, a core protein of 13,500 MW, and a capsid protein of 7,700 MW. The core protein was missing in incomplete hemagglutinating particles. This type of information is essential to development of effective vaccines.

SUMMARY OF THE SECOND FIVE YEARS

During the second 5 years, research emphasis was placed on rabies, dengue and dengue hemorrhagic fever, Japanese encephalitis, and other arboviruses. Much attention was given to the mosquito, *Culex tritaeniorhynchus*. A new, inactivated rabies virus vaccine was developed in human diploid cells, and there were important new developments on immunogathogenic mechanisms as a cause of early deaths in rabbits. The production of rabies vaccine in many countries depends on the use of neural tissues. Hence, the new vaccine represented an important scientific advance. Other viruses were isolated in Africa and were identified as members of the rabies-virus complex.

The pathogenesis of dengue hemmorrhagic fever is improperly understood. In pursuit of a dengue virus vaccine, sensitive mutants of the virus have been developed. *Aedes albopictus* taken from different geographic areas were shown to demonstrate different susceptibilities to dengue type 2 virus. Genetically differing strains of *Culex tritaeniorhynchus*, a major vector of Japanese encephalitis virus in Asia, have been developed. These studies are aimed at finding viable strains which would be poor vectors. Yet, they might compete genetically with wild populations and replace the wild vector populations with inefficient vectors.

SUMMARY OF THIRD FIVE YEARS

The mechanism of dengue hemorrhagic fever/shock syndrome remains unclear. Severity of disease is not related to transmission by certain vector species. Immune enhancement of dengue virus infection was well established, which suggests that entry of dengue virus antibody complexes into mononuclear phagocytes occurs via Fc receptors on these cells. Products are then released which alter vascular permeability. Several structural and non-structural viral proteins were described for St. Louis and dengue viruses. The mechanism of replication of flaviviruses is different from that of alpha viruses. In flaviviruses, ribosomes recognize internal initiation sites on genomic messenger RNA. This is in contrast to alpha virus translation, which begins at terminal sites, with production of precursors that are subsequently cleaved.

Vector infection studies **showed** the relationship of the midgut barrier to vector competence. Other studies with California group bunyaviruses and their *Aedes* vectors **revealed** that transovarial transmission occurs commonly. Transovarial transmission of Japanese and St. Louis encephalitis, and dengue viruses occur in several species in the laboratory, but their importance in the field has not been established. Korean hemorrhagic fever (hemorrhagic fever with renal syndrome) is present in the wild rodent *Apodemus agrarius*. Cells from these rodents were used to isolate the agent from humans. The disease is now known to occur in Japan.

The structure of rotaviruses was demonstrated. Human rotavirus type 2 was successfully propagated in cell cultures. Epidemiological studies have shown rotaviruses, adenovirus and non-cultivatable 27nm viruses responsible for acute viral gastroenteritis in humans. The viral infections were detected by ELISA and immunoelectron microscopy. Rotaviral infections were most common. Rotaviral antibody prevalence was shown to increase with age. Sporadic outbreaks of Norwak-type (27nm particle) occurred in the United States.

The human diploid cell inactivated rabies virus vaccine consistently produces antibodies in human recipients. Tests of an oral rabies vaccine for animal administration resulted in antibody production. A guinea pig model system for post-exposure testing of rabies vaccine efficacy was developed. Oligonucleotide fingerprints showed that fixed rabies virus RNA differs from wild strains. A monoclonal antibody system showed that the glycoprotein antigens were diverse. Strains from the same geographic area and species of origin have similar patterns of reactivity. Natural antigenic variation may explain failures of post-exposure treatment of rabies. Cell to cell spread of rabies virus occurs by budding of virus from one cell directly into the cytoplasm of an adjacent one.

PROGRESS IN PRIORITY AREAS

Dengue and Other Arboviruses Including Korean Hemorrhagic Fever

Progress has been steady in reaching a more complete understanding of the molecular biology of arboviruses. Insight into the process of replication, production of structural and non-structural viral proteins, and the genetics of antigenic variation and cell and tissue tropisms will be necessary to understand the mechanism by which viruses infect cells, produce disease, and elicit immune responses in their vertebrate hosts. Such data will clarify the mode of transmission by their arthropod vectors.

Studies have focused on alpha virus transcription. Viral minus strand RNA is the template of synthesis for two species of plus strand RNA—the 49S virion RNA and 26S messenger RNA. Minus strand synthesis is required to amplify virion RNA in infected cells. Minus strand RNA synthesis ceases at 3–4 hours post-infection even when viral non-structural proteins that comprise the viral RNA dependent RNA polymerase continue to be synthesized at a high rate. This phenomenon appears to be due to "cis" acting viral function restricted to a particular transcription complex and is not freely diffusable.

Complementary DNA (cDNA) corresponding to the genes and coding viral glycoproteins of two temperature sensitive-mutants of Sindbis virus were sequenced. Work with mutants defective in transport of the glycoproteins from the rough endoplasmic reticulum to the plasma membrane indicates that the signal for transport is specified by the protein itself. This suggests that alinear domain in the protein is not responsible for correct intracellular transport. The three dimensional protein conformation may be very important. The outcome of virus replication in arthropod cells frequently differs considerably from that of vertebrate cells. Hence, a fundamental understanding of virus-arthropod cell interactions is important. Studies with *Aedes albopictus* cells deficient in the transport of adenosine and other nucleosides and certain of their analogs, suggests that in mosquito cells, as in mammalian cells, a single system transports all nucleosides. The polypeptides of dengue type 2 virus were studied in mammalian and mosquito cells. Eleven presumably virus specific proteins were found, ranging in molecular weight from 12K to 100K. More than 90% of the virion RNA-stimulated protein was suppressed by cap analogs, which indicates that translation is initiated at cap-dependent sites. The mosquito system generates fewer discrete products than do mammalian systems. This suggests that there is less premature (non-specific) termination of translation in this system.

Other studies have investigated factors involved in persistent viral infection of *Ae. albopictus* cells. These cells have been infected with banzi virus, a flavivirus, and secrete a factor of low molecular weight that inhibits replication of this virus either in mosquitos or in mammalian cells. Inhibition is virus-specific and does not affect the replication of heterologous viruses. This factor is initially detectable when acute infection of mosquito cells evolves to a persistent infection. This factor neither hinders the attachment of the virus to mosquito cells, nor prevents penetration and uncoating.

Considerable progress has been made in the understanding of bunyavirus genetics. Bunyaviruses have three RNA species designated L (large), M (medium), and S (small). Viral recombination can be obtained only between members of the same serogroup. LaCrosse virus isolates have been categorized into more than three genotypic varieties, but intertypic reassortants have been shown to occur only occasionally. Analysis of bunyaviruses showed that the S RNA codes for nucleoprotein and a non-structural protein. The M RNA codes for two glycoproteins and a second non-structural protein. The L RNA probably codes for a large protein present in virions believed to be the viral transcriptase. The M RNA determines both the transmission capabilities in the arthropod vector and the viral induced pathogenesis in vertebrate hosts. Monoclonal antibody studies with LaCrosse virus revealed that eight antigenic sites occur on the G1 glycoprotein. It appears that binding of an antibody to one of these antigenic sites allosterically alter the glycoprotein so that other sites become more accessible for antibody binding. High frequency reassortment occurs in another virus with a segmented genome, i.e. Colorado tick fever virus of the orbivirus group. High frequency reassortment was demonstrated *in vitro* and in *Dermacentor andersoni* ticks, the natural vector of this virus. Reassortment was nearly random, although possibly some linkage occurred especially *in vivo*. Segment reassortment allows for easy transfer of genes in nature and for genetic heterogeneity.

Considerable progress led to better understanding of the occurrence and viral characteristics of the agents which cause Korean hemorrhagic fever (hemorrhagic fever with renal syndrome). In work reported at joint panel meetings, Hantaan virus, the designated etiologic agent of Korean hemorrhagic fever, was isolated in cell culture from clinical cases and wild rodents in Korea and Japan. This virus is a bunyavirus. These observations stimulated work elsewhere; related viruses have been islolated from rodents in the United States and Europe. These findings suggest a close association with rodents and worldwide distribution. Biochemical and antigenic analysis of these isolates indicate that they form a complex designated Hantaan virus group.

Research sponsored by the U.S. panel led to better understanding of Old World and African arenaviruses. GP-1 and GP-2 antigens of LCM and Lassa and Mozambique were studied. This work revealed definitive evidence of the conservation of genetic information coding for GP-2 among these three viruses and their close evolutionary links.

Studies on pathogenesis of LCM virus showed that antigens are expressed in the brains of acutely infected mice, but the viral GP antigen diminishes to undetectable levels during the progress of persistent infection. Such selective expression of viral glycoprotein may explain how virus is able to escape immunorecognition *in vivo*, and establish persistent infection.

Progress in molecular arbovirology now provides useful tools for study of arbovirus pathogenesis. St. Louis encephalitis and LaCrosse viruses attach at the neuro-muscular junction which may indicate the site of entry of these viruses into the peripheral nervous system. There are specific receptor sites at synaptic regions, some of which are related to neurotransmitters. The G1 glycoprotein of LaCrosse virus carries an attachment site to fibroblasts and erythrocytes. Also, differences between virulence of LaCrosse and Tahyna viruses may reside in their ability to replicate in striated and cardiac muscle. This activity resides in the M RNA segment, as a determinant of LaCrosse virus virulence.

New approaches were **applied** to improvement of arbovirus diagnosis. Monoclonal antibodies and ELISA tests were adapted to systems for early and rapid diagnosis and identification of viruses. ELISA functioned well for Rift Valley Fever and correlated with neutralization tests of post-vaccination human sera. The ELISA was broadly cross-reactive with human and mouse immune sera to other phleboviruses. An 8-minute latex agglutination test, used in rubella diagnosis, adapted to arboviruses is a more rapid and sensitive reaction in pools of mosquitoes. Correlation was excellent with eastern equine encephalitis and Highlands J viruses when compared to conventional techniques applied to field specimens. The ELISA technique also detected LaCrosse virus antigen in one infected mosquito in a pool of 100 individuals. It was specific and distinguished between closely related California group viruses. This test is rapid, specific and sensitive.

A variety of research projects have provided better understanding of the relationship of arthropod-borne viruses and their vector mosquitoes. To explain the absence of yellow fever virus in southeast Asia, populations of *Ae. aegypti* from this region were tested for susceptibility to yellow fever virus. Mosquitoes from this region of the world were as susceptible and competent vectors as most *Ae. aegypti* populations from yellow fever endemic areas from Africa and South America. Thus, geographic differences and vector competence do not appear to be responsible for the absence of yellow fever in southeast Asia. In other studies on yellow fever virus transmission, *Haemagogus equinus* which fed on yellow fever virus in blood-soaked pledgets were less easily infected than those fed on viremic monkeys. These mosquitoes captured from the field late in the season were slightly more easily infected than early season mosquitoes captured at the same site. This phenomenon would favor amplification as an epizootic builds and transmission accelerates. Interestingly, transovarial transmission of yellow fever virus was demonstrated experimentally in *H. equinus*.

The vector efficiency of *Culex pipiens* and *Cx. quinquefasciatus* was markedly different when tested with St. Louis encephalitis viral strains from four different geographical regions. Overall, however, *Cx. pipiens* is a better vector of St. Louis encephalitis virus than is *Cx. quinquefasciatus*. Other experiments indicated that five strains of St. Louis encephalitis virus isolated in California from 1950 to 1963 were transmitted by *Cx. quinquefasciatus* more efficiently than were two strains isolated in the 1970s. In other evaluations, female *Cx. tarsalis* collected in May were approximately tenfold more susceptible to infection with western equine encephalitis virus than were those collected in mid-summer. Attempts were made to introduce an autosomal genetic mutation into a field population of *Cx. tarsalis* at an isolated site, to determine if this gene would persist. Phenotypic frequency declined rapidly following the vernal increase of the native mosquito population, but males heterozygous for the trait were recovered throughout the summer and early fall.

Other studies with dengue virus and aedine species other than *Ae. aegypti* showed a new world species, *Aedes (Gymnometopa mediovitatus)*, occupies the same general ecologic niche as *Ae. aegypti*, and bites man readily. It is susceptible to oral infection with dengue 1 virus and is more susceptible to dengue 2 virus infection than a highly susceptible strain of *Ae. aegypti*. Of particular interest is the demonstration of transovarial transmission of dengue virus in *Ae. mediovitatus*. It showed the highest overall filial infection rates **observed** for any flavivirus including recorded rates for *Aedes aegypti* with all four dengue types.

Results from other experiments demonstrate transovarial transmission of Japanese encephalitis virus in *Cx. tritaeniorhynchus*; results were erratic. Filial infection rates were relatively high but widely variable, ranging from 0 to 37%. This suggests that transovarial transmission may be influenced by vector genetics and one might select subpopulations of mosquitoes which show high transovarial transmission rates. The most complete understanding of transovarial transmission came from experiments with LaCrosse and other California group bunyaviruses. Transovarial transmission of LaCrosse virus was highly efficient in *Ae. triseriatus*, and is the mechanism by which the virus overwinters and persists for long periods in endemic foci. However, transovarial transmission does not occur in all of the progeny. Venereal transmission from infected male mosquitoes to susceptible females, and horizontal transmission to and from small forest mammals supplements the basic maintenance cycle in transovarially infected mosquitoes. Movement of the vector mosquito is relatively limited and may explain the occurrence of the virus in sharply delineated foci. Recent studies showed some movement across open fields and along brushy fence lines. These movements probably account for local recolonization of areas by *Ae. triseriatus*, and probably serve to establish persistent LAC virus foci in close contact with humans.

Host-virus-vector studies were conducted in the neotropics. The relationship of Venezuelan equine encephalitis (VEE) virus to vector mosquitoes has been ascertained. Epidemic strains of VEE virus cannot cross the mesenteronal barrier in *Cx. (Melanoconion) taeniopus* from Central America. This species is the natural vector of the endemic IE strain of VEE virus, for which there is no mesenteronal barrier. Virus disseminates throughout the mosquito and is efficiently transmitted by it. Other tropical studies documented the occurrence of St. Louis encephalitis (SLE) virus in Panama. The virus occurs in birds and is efficiently transmitted by the same species of mosquitoes that transmitted in temperate North America (*Cx. pipiens quinquefasciatus*) experimentally. Yet there is not evidence of human illness in this area, unlike the situation in more temperate zones.

Viral Gastroenteritis

Progress in classification and understanding of rotaviruses and their structure, composition, and function was rapid. Swift progress was largely due to stimulating research reports presented at joint panel meetings, constant communication between U.S. and Japanese rotavirologists, and active collaboration with the exchange of information, reagents and scientists. A review of the last 5 years of joint panel presentations shows many major advances. A cell culture system was developed and rapidly employed to isolate rotaviruses and characterize them. A refinement of the cell culture system employing trypsin treatment has resulted in high virus yields. This large scale *in vitro* cultivation system permitted characterization of growth and analysis of purified structural polypeptides. Five proteins with molecular weights ranging from 41K–116K are components of the inner shell and four proteins with molecular weights ranging from 27K–60K are components of the outer shell. Rotavirus neutralizing antigen contains type specific and type common determinants. **Both** segment 3 and segment 9 products are involved in type specific neutralization and the function and complementarity of the 11 rotavirus segments have been demonstrated; reassortment occurs. A mouse model to study homologous and heterologous immune responses to rotavirus infection is being developed.

Other viral agents which cause gastroenteritis are being isolated, and characterized and their epidemiologies studied. The Norwak agent is a common cause of outbreaks of gastroenteritis in the United States; by early childhood antibody to this agent is found throughout the world in high frequency. The newly described Snow Mountain agent is being characterized. It is morphologically similar to Norwak and Hawaii agents but is antigenically distinct. A sensitive radioimmunoassay for Snow Mountain agent detects virus particles and soluble antigens in stool specimens from acutely ill patients. The prevalence of Snow Mountain agent antibody appears to be similar to that of Norwak agent. Antibody is acquired late in childhood; prevalence is approximately 60% in selected adult populations through the United States.

Rabies

The development of human diploid cell inactivated rabies virus vaccines, and more effective killed and live attenuated animal vaccines stimulated interest in evaluation of rabies virus antigens and the relationship of these antigens to human protection. The rabies virus glycoprotein gene indicates that a polypeptide of

524 amino acids is translated from messenger RNA. The first 19 amino acids form a single polypeptide cleaved from the glycoprotein. Amino acid sequence predicts three carbohydrate attachment sites on the glycoprotein located on the N terminal (exposed) side of the proposed transmembrane segment. A relatively long sequence of 44 amino acids which extend inward from the presumptive transmembrane domain to the C terminal may provide a site for strong interaction with the internal viral matrix and nucleocapsid proteins. Antigenic studies show the existence of at least three distinct sites on the rabies virus glycoprotein. Epitope maps were constructed that operationally define antigenic sites on virion glycoprotein of two fixed rabies virus strains, ERA and challenge virus standard (CVS). These studies showed a minimum of four antigenic sites in each strain although the patterns of complementary antibody-variant virus groups were not identical for the two strains. Three of the antigenic sites in CVS and four in ERA strains appeared to be functionally independent. Mutations in the glycoprotein gene of rabies viruses possessing antigenically distinct glycoprotein were mapped by DNA sequence analysis. This is based on monoclonal antibody reactions. Mutations in the nucleotide sequence, resulting in amino acid substitutions that alter the binding of the neutralizing antibodies of the glycoprotein, were identified in ERA and CVS rabies strain variants. A **mutation** was located in the non-pathogenic variant glycoprotein gene which confirmed the amino acid substitution at position 333 of the molecule. Identification of a mutation presumably responsible for a change in molecular conformation appeared to alter glycosylation in a CVS variant at one carbohydrate attachment site. Other antigenic evaluations were made with Mokola, a related rabies virus from Zimbabwe. This virus was responsible for the first rabies-like epidemic in dogs and cats. Conventional rabies vaccines do not protect against infection with Mokola virus.

Viral and immune components involved in rabies pathogenesis are under study. The role of rabies viral antigens in the cytotoxic T lymphocyte (CTL) response was evaluated in mice. Rabies virus glycoprotein induces CTL but only when incorporated into virosomes. One small region of the glycoprotein accounts for this stimulatory activity. Some rabies strains cause generalized suppression of T lymphocyte response, with similarities to acquired immunodeficiency syndrome (AIDS) in humans. Specific rabies virus recognition sites on the surface of susceptible cells were characterized. Discrete binding sites are on the plasma membrane of cells. Purified rabies glycoprotein alone binds non-specifically to cells.

The spread of rabies virus through the nervous system was studied. Single rabies virus-like particles were frequently found on the post-synaptic side of the neuromuscular junction either free or enclosed in small vacuoles located at the periphery of the junctional folds. Later, rabies virus antigen was detected by immunoelectron microscopy in neuromuscular junctions and in axons of the peripheral nervous system. Degenerative changes occurred 5 days after inoculation involving the pre-synaptic element. Morphologic disorder of junctional folds themselves **were found** in several instances where the nerve terminal had undergone complete dissolution. Immune and/or phagocytic cells were detected in the extracellular spaces at the neuromuscular junction. In addition, there were macrophages in developing cells that had undergone degenerative changes. In the central nervous system, neuronal cell bodies were intact with few immune or phagocytic cells nearby. However, cellular processes degenerated with massive accumulation of matrices and intact particles. In many instances, immune and/or phagocytic cells surrounded these processes. Viral proteins occur on the surface of neurons, limited to synpatic regions often near budding virions. In dentritic processes, isolated viral proteins are apparently transported in association with microtubules.

To understand the dynamics of rabies virus-cell interactions, studies were directed to defective interfering particles. These particles are commonly associated with cells persistently infected with this virus. These persistently infected cells constantly produce large amounts of intracellular viral cores that contain viral RNA genes with a coat protein associated with viral replication enzymes. Only a small percentage of full-size infectious rabies virions are produced. This confirms that defective interfering particle nucleocapsids regulate *in vitro* rabies replication during years of persistence. The relative proportion of virus nucleocapsid and DI particle nucleocapsid within persistently infected cells constantly changes. Replication of infectious virus is always lowest when DI particle nucleocapsid levels are at the peak of their cycle, and vice versa.

FUTURE GOALS

Dengue and Other Arboviruses

The classic form of dengue fever and hemorrhagic fever/shock syndrome, a current problem in southeast Asia, has appeared in the American Tropics. Although there are two working hypotheses to explain hemorrhagic fever with shock, additional work is needed on the fundamental virology and immunopathology of this important disorder. Japanese encephalitis virus continues to cause epidemics in substantial numbers in southeast Asia and China. St. Louis encephalitis and other arthropod-borne viruses are a human health problem in the Western Hemisphere. The molecular biology, pathogenesis and epidemiology of these viruses must be clarified in order to provide fundamental information. This is required before effective control measures can be developed and implemented.

Development of effective vaccines requires an understanding of the synthesis of structural and non-structural viral proteins, their action in virus replication and their interaction with immune systems. An understanding of pathogenesis necessitates further research on cellular receptors and replicative mechanisms as the basis of tissue tropism and damage, and virulence factors.

The dissemination and maintenance of arboviruses in nature must rest on a deeper understanding of host-virus-vector relationships. Studies on infection of arthropods by arboviruses, and vector genetics should advance relatively rapidly, depending in large part on newer molecular techniques directed toward answering questions. The role of transovarial transmission needs to be extended from the laboratory to the field with respect to its significance in natural maintenance of flaviviruses, alpha viruses and bunyaviruses. The dynamics of vector and host populations needs to be better quantified, as does further measurement of environmental effects on arboviral infection in mosquito vectors and vertebrate hosts.

Viral Gastroenteritis

The importance of rotaviruses and Norwalk-like agents as etiologic agents in viral gastroenteritis is sufficiently well documented to justify vigorous research aimed at development of vaccines. Additional work is needed on the structural and non-structural proteins of these viruses, and their antigenic specificity. Further work is needed on immunology of the gut, in order to develop effective mechanisms to deliver antigens to immunoresponsive sites. Antigens must be in a form that will stimulate immunity of sufficient duration and breadth to prevent clinical illness. Development of feasible animal models will be required. Further molecular studies on rotavirus recombination may prove essential in developing a spectrum of antigens that can avoid vaccine failures due to viral recombination. Additional work on the characterization of Norwalk-like agents is also needed. Epidemiological studies must be directed to viral gastroenteritis in the developing (especially tropical) world in order to determine the specific etiology, times of occurrence, morbidity and mortality. This data will help clarify whether candidate vaccines developed for the industrial countries are suitable for prevention of disease in those developing countries, with identified populations at highest risk.

Rabies

Rabies is an important public health problem in North America and is a significant economic problem in domestic animals in developing countries, especially in Latin America. Further evaluation of antigenic variation of rabies virus glycoproteins is essential for development of vaccines that provide good protection for reasonable periods of time. The development of safe and effective oral vaccines for dogs and cats and for wildlife is as urgently needed as ever. Research, suspended due to an unfortunate laboratory accident, should be resumed and pursued with vigor. Development of small-scale, simple rabies vaccine systems for production in developing countries merits serious consideration. Additional studies on the pathogenesis of rabies infection is needed. In spite of better knowledge of the mechanism by which rabies virus reaches and disseminates in the nervous system, such work should be expanded. This will provide the insight necessary for improved therapy and better information on the illness itself. The development of effective chemotherapy is a long term goal. Advances will require a more complete understanding of the biochemistry of rabies virus infection, cellular interactions, further insights into pathogenesis caused by virus replication and host-immune reponses to it.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institutions	*Title*
Barry Beaty Colorado State University Fort Collins	Bunyavirus reassortants in vectors
David H. Bishop University of Alabama Birmingham	Genetic and molecular studies of bunyaviruses
Margo A. Brinton Wistar Institute Philadelphia	Cellular gene product modulates flavivirus RNA synthesis
Dennis T. Brown University of Texas Austin	Arbovirus growth in cultured insect cells Development of a virus membrane
Rise K. Cross Sloan-Kettering Institute for Cancer Research New York City	Togavirus RNA replication: role of viral proteins
Gene DeFoliart University of Wisconsin Madison	Transmission and control of California-group viruses

Investigator and Institution	*Title*
William F. Scherer and Robert W. Dickerman Cornell Medical School New York City	Ecology of endemic and epidemic strains of Venezuelan equine encephalitis virus
Raphael Dolin University of Rochester Rochester, N.Y.	Studies of Norwalk-like agents of viral gastroenteritis
Harry B. Greenberg Stanford University Palo Alto	Viral gastroenteritis: basis of virulence and protection
Paul Grimstad University of Notre Dame Notre Dame, Indiana	Ecology of vectors of Jamestown Canyon virus
James Hardy and William C. Reeves University of California Berkeley	Ecology and control of arboviruses
John Holland University of California La Jolla	Synthesis in normal and virus-infected cells
Robert E. Johnston North Carolina State University Raleigh	Early Sindbis proteins in lytic and persistent infections
Laura Kingsford California State University Long Beach	Molecular mechanisms of virus neutralization
Hilary Koprowski Wistar Institute Philadelphia	Studies of rabies and rabies virus antigens
Andrew Main Yale University New Haven	World reference center for arboviruses
Neal Nathanson University of Pennsylvania Philadelphia	Molecular basis of bunyavirus virulence
Robert Ramig Baylor School of Medicine Houston	Genetics of the rotavirus
Leon Rosen University of Hawaii at Manora Honolulu	Transovarial transmission of encephalitis viruses and dengue viruses; also Aedine eggs, Toxorhyncites, and arboviruses

Investigator and Institution	*Title*
Donald Rubin University of Pennsylvania Philadelphia	Reovirus: gastrointestinal infection and immunity
Dorothea Sawicki Medical College of Ohio Toledo	Regulation of alphavirus transcription
R. Walter Schlesinger and Victor Stollar Rutgers University	Arbovirus replication in mosquito and vertebrate cells
Robert Schloemer Indiana University Indianapolis	Persistent viral infection in mosquito cells
Robert Shope Yale University New Haven	Control of arbovirus infections in man
James Strauss California Institute of Technology Pasadena	Togavirus replication strategies and evolution
Gregory Tignor Yale University New Haven	Rabies serogroup viruses: immunopathology
William H. Wunner Wistar Institute Philadelphia	Cell membrane receptors for rabies virus and nucleotide sequences encoding antigenic sites
Thomas M. Yuill University of Wisconsin Madison	Wildlife reservoirs of arboviruses

BIBLIOGRAPHY

Dengue

Cleaves, G.R., T.E. Ryan, and R.W. Schlesinger. Identification and characterization of type 2 dengue virus replicative intermediate and replicative form RNAs. *Virology 111*:73–83, 1981.

Rosen, L., D.A. Shroyer, R.B. Tesh, J.E. Freier, and J.C. Lien. Transovarial transmission of dengue viruses by mosquitoes: *Aedes albopictus* and *Aedes aegypti*. *Amer. J. Trop. Med. Hyg. 32*:1108–1119, 1983.

Rosen, L. The use of *Toxorhynchites* mosquitoes to detect and propagate dengue and other arboviruses. *Am. J. Trop. Med. Hyg. 30*:177–183, 1981.

Schlesinger, R.W. General Review of Dengue and Dengue Hemorrhagic Fever, in "Dengue Hemorrhagic Fever 1981", Proceedings of the First ICMR Seminar International Center for Medicine (S. Hotta, ed.), 13-31, 1981.

Shope, R.E. Overview: Dengue threat in the 1980's. In: *Proceedings of the International Conference on Dengue/Dengue Haemorrhagic Fever*. T. Pang and R. Pathamnathan (editors), pp. 494–506. Univ. Malaya, Kuala Lumpur, Malaysia, 1983.

Tesh, R.B. Undifferentiated fevers: dengue, phlebotomus fever, West-Nile fever, Rift Valley fever and fevers caused by alphaviruses. *In*: Textbook of Medicine, 16th edition. J.B. Wyngaarden and L.H. Smith, Jr., editors. *W. B. Saunders Co.*, Philadelphia, pp. 1572–1676, 1982.

Vezza, A.C., L. Rosen, R. Repik, J. Dalrymple, and D.H.L. Bishop. Characterization of the viral RNA species of prototype dengue viruses. *Am. J. Trop. Med. Hyg. 29*:643–652, 1980.

Other Arboviruses

Aaskov, J.G., C.E.A. Davies, M. Tucker, and D. Dalglish. Effect on mice of infection during pregnancy with three Australian arboviruses. *Am. J. Trop. Med. Hyg. 30*:198–203, 1981.

Adams, R.H., and D.T. Brown. Inhibition of Sindbis virus maturation after treatment of infected cells with trypsin. *J. Virol. 41*:692, 1982.

Ainsley, R.W., S.M. Asman, and R.P. Meyer. The optimal radiation dose for competitive males of *Culex tarsalis* (Diptera: Culicidae). *J. Med. Ent. 17*:122–125, 1980.

Beaty, B.J., B.R. Miller, R.E. Shope, E.J. Rozhon, and D.H.L. Bishop. Molecular basis of Bunyavirus per os infection of mosquitoes: Role of the middle-sized RNA segment. *Proc. Natl. Acad. Sci. 79*:1295–1297, 1982.

Beaty, B.J., D.H.L. Bishop, M. Gay, and F. Fuller. Interference between Bunyaviruses in *Aedes triseriatus* mosquitoes. *Virology 127*, in press, 1983.

Beaty, B.J., F. Fuller, and D.H.L. Bishop. Bunyavirus gene structure function relationships and potential for RNA segment reassortment in the vector: La Crosse and snowshoe hare reassortant viruses in mosquitoes. *Proc. Intl. Symp. California Serogroup Viruses*. C. Calisher and W. Thompson (eds.) *A. R. Liss*, New York, pp. 119-128, 1983.

Beaty, B.J., D.H.L. Bishop, M. Gay, and F. Fuller. Interference between Bunyaviruses in *Aedes triseriatus* mosquitoes. *Virology 127*:83–90, 1983.

Bell, J.R., and J.H. Strauss. *In vivo* N-terminal acetylation of Sindbis virus proteins. *J. Biol. Chem. 252*:8006–8011, 1981.

Bia, F.J., G.F. Thompson, A.J. Main, C.K.Y. Fong, and G.D. Hsiung. Western equine encephalitis. *JAMA 244*:367–373, 1980.

Bishop, D.H.L., C.H. Calisher, J. Casals, M.P. Chumakov, S.Y. Gaidamovich, C. Hannoun, D.K. Lvov, I.D. Marshall, N. Oker-Blom, R.F. Pettersson, J.S. Porterfield, P.K. Russell, R.E. Shope, and E.G. Westaway. Bunyaviridae. *Intervirology 14*:125–143, 1981.

Bishop, D.H.L., F. Fuller, H. Akashi, B. Beaty, and R.E. Shope. The use of reassortant Bunyaviruses to deduce their coding and pathogenic potentials. *Proc. 28th Oholo Conference—From Gen to Pathogen. M. Nijhoff*, Israel, pp. 49–60, 1984.

Boromisa, R.D., and P.R. Grimstad. Ecology of vectors of Jamestown Canyon virus: Seasonal succession of hematophagous diptera at Kingsbury State Fish and Wildlife Area, LaPorte County, Indiana. *Indiana Academy of Science, Proceedings* 92:239–245, 1983.

Brown, S.E., and D.L. Knudson. Characterization of invertebrate cell lines. IV. Isozyme analyses of dipteran and acarine cell lines. *In Vitro 18*:347–350, 1982.

Burkot, T.R., and G.R. DeFoliart. Bloodmeal sources of *Aedes triseriatus* and *Aedes vexans* in a southern Wisconsin forest endemic for LaCrosse encephalitis virus. *Am. J. Trop. Med. Hyg. 31(2)*:376–381, 1982.

Cash, P., L. Hendershot, and D.H.L. Bishop. The effects of glycosylation inhibitors on the maluration and intracellular polypeptide synthesis induced by snowshoe hare bunyavirus. *Virology, 103*:235–240, 1980.

Clerx, J.P., J. Casals, and D.H.L. Bishop. Structural characteristics of nairoviruses (genus *Nairovirus*, Bunyaviridae). *J. Gen. Virol. 55*:165–178, 1981.

Clewley, J.P., and D.H.L. Bishop. Oligonucleotide fingerprinting of viral genomes. In: Newer Techniques in practical virology (ed. C. Howard), *Alan Liss Inc.* New York, *in press*, 1980.

DeFoliart, G.R. *Aedes triseriatus:* vector biology in relationship to the persistence of LaCrosse virus in endemic foci. *In* C.H. Calisher, W.H. Thompson, (eds.), *California Serogroup Viruses*, New York: Alan R. Liss, Inc., pp. 89-104, 1983.

Gentsch, J.R., and D.H.L. Bishop. The genetic recombination potential of bunyaviruses, Proc. 6th FEMS Symposium: Arboviruses in the Mediterranean countries, 1980.

Gentsch, J.R., E.J. Rozhon, R.A. Klimas, L.H. El Said, R.E. Shope, and D.H.L. Bishop. Evidence from recombinant bunyavirus studies that the mRNA gene products elicit neutralizing antibodies. *Virology*, *102*:190-204, 1980.

Gomatos, P.J., L. Kaariainen, S. Keranen, M. Ranki, and D.L. Sawicki. Semliki Forest virus replication complex capable of snythesizing 42S and 26S nacent RNA chains. *J. Gen. Virol. 49*.

Gonzalez-Scarano, F., R.E. Shope, C.H. Calisher, and N. Nathanson. Monoclonal antibodies against the G1 and nucleocapsid proteins of LaCrosse and Tahyna viruses. In: *California Serogroup Viruses*. C.H. Calisher and W.H. Thompson (editors). pp. 145-156. Alan R. Liss, New York, 1983.

Gonzalez-Scarano, F., R.E. Shope, C.H. Calisher, and N. Nathanson. Characterization of monoclonal antibodies against the G1 and N proteins of LaCrosse and Tahyna, two California serogroup bunyaviruses. *Virology*, *120*:42-53, 1982.

Grimstad, P.R. Mosquitoes and the incidence of encephalitis. Advances in Virus Research, *Academic Press*, New York. *Vol. 28*:357-438, 1983.

Grimstad, P.R., and L.D. Haramis. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus III: Enhanced oral transmission by nutrition deprived mosquitoes. *J. Med. Entomol. 21*: (in press) 1984.

Hardy, J.L., L. Rosen, L.D. Kramer, S.B. Presser, D.A. Shroyer, and M.J. Turell. Effect of rearing temperature on transovarial transmission of St. Louis encephalitis virus by mosquitoes. *Am. J. Trop. Med. Hyg. 29*: 715-719, 1980.

Hildreth, S.W., B.J. Beaty, J.M. Meegan, C.L. Frazier, and R.E. Shope. Detection of LaCrosse arbovirus antigen in mosquito pools: application of chromogenic and fluorogenic enzyme immunoassay systems. *J. Clin. Micro. 15*:879-884, 1982.

Hildreth, S.W. and B.J. Beaty. Application of enzyme immunoassays (EIA) for the detection of LaCrosse viral antigens in mosquitoes. *Proc. Intl. Symp. California Serogroup Viruses*. C. Calisher and W. Thompson (eds.) *A.R. Liss*, New York, pp. 303-312. 1983.

Hildreth, S.W., and B.J. Beaty. Detection of Eastern equine encephalitis virus within mosquito pools. I.A. laboratory study. *Am. J. Trop. Med. Hyg.*, in press. 1984.

Hildreth, S.W., B.J. Beaty, H.K. Maxfield, R.F. Gilfillan, and B.J. Rosenau. Detection of eastern equine encephalitis virus within mosquito pools. II. Retrospective field test of the enzyme immunoassay. *Am. J. Trop. Med. Hyg.*, in press. 1984.

Horodyski, F.M., and J.J. Holland. Viruses isolated from cells persistently infected with vesicular stomatitis virus show altered interactions with defective interfering particles. *J. Virol. 36*: 627-631, 1980.

Kingsford, L. Structure and Biological Function of La Crosse G1 Glycoprotein *in* California Serogroup Viruses. Edited by C.H. Calisher and W.H. Thompson, *Alan R. Liss Inc.*, New York, 1983.

Knudson, D.L. Genome of Colorado tick fever virus. *Virology*, *112*:361-364, 1981.

Knudson, D.L., W.K. Butterfield, R.E. Shope, T.E. Walton, and C.H. Campbell. Electrophoretic comparison of the genomes of North American bluetongue viruses, one Australian bluetongue virus, and three other related orbiviruses. *Vet. Microbiol. 7*:285-293, 1982.

Knudson, D.L., R.B. Tesh, A.J. Main, T.D. St. George, and J.P. Digoutte. Characterization of the Palyam virus serogroup (Reoviridae: *Orbivirus*). Manuscript.

Knudson, D.L., and R.E. Shope. Overview of the Orbiviruses. In: *International Symposium on Bluetongue and Related Orbiviruses*. T.L. Barber and M.M. Jochim (editors). Asilomar, California, January 16-20, 1984.

Kowal, K.J. and V. Stollar. Differential sensitivity of infectious and defective-interfering particles of Sindbis virus to UV-irradiation. *Virology 103*:149-158, 1980.

Kowal, K.J. and V. Stollar. Temperature sensitive host dependent mutants of Sindbis virus. *Virology 114*:140-148, 1981.

Kramer, L.D., J.L. Hardy, S.B. Presser, and E.F. Houk. Dissemination barriers for western equine encephalomyelitis virus in *Culex tarsalis* infected after ingestion of low viral doses. *Am. J. Trop. Med. Hyg. 30*:194-201, 1981.

Main, A.J. Arbovirus surveillance in Connecticut. IV. Bunyamwera *Mosq. News, 41*:490-494, 1981.

Main, A.J. "Eastern equine encephalomyelitis." *In* Geographical Distribution of Animal Viral Diseases, edited by S. Odend'hal. *Academic Press*. 1982.

Main, A.J. "Western equine encephalomyelitis." *In* Geographical Distribution of Animal Viral Diseases, edited by S. Odend'hal. *Academic Press*. 1982.

Main, A.J. "Powassan virus" *In* Geographical Distribution of Animal Viral Diseases, edited by S. Odend'hal. *Academic Press*. 1982.

Malinoski, F., and V. Stollar. Inhibitors of IMP dehydrogenase prevent Sindbis virus replication and reduce GTP levels in *A. albopictus* cells. *Virology 110*:281-291, 1981.

Malinoski, F., and V. Stollar. Inhibition of Sindbis virus replication by ribavirin: Influence of cultural conditions and of the host cell phenotype. *Antiviral Res. 1*: 287-299, 1981.

Malinoski, G., and V. Stollar. Inhibition of Sindbis virus replication in *Aedes albopictus* cells by Virazole (ribavirin) and its reversal by actinomycin: A correction. *Virology 102*:473–476, 1980.

Mather, T.N., and G.R. DeFoliart. Effect of host blood source on the gonotrophic cycle of *Aedes triseriatus*. *Am. J. Med. Hyg 32(1)*:189–193, 1983.

Mather, T.N., and G.R. DeFoliart. Repellency and initial toxicity of Abate and Dursban formulation to *Aedes triseriatus* in oviposition sites. *Mosq. News 43(4)*:474–479, 1983.

Mather, T.N., and G.R. DeFoliart. Conditions for increased insemination rates in caged *Aedes triseriatus* (Diptera: Culicidae) reared from field-collected eggs. *Am. J. Trop. Med. Hyg 33(4)* 731–735, 1984.

Mather, T.N., and G.R. DeFoliart. Dispersion of gravid *Aedes triseriatus* (Diptera: Culicidae) from woodlands into open terrain. *J. Med. Entomol. 21(4)*:384–91, 1984.

Mathur, A., K.L. Arora, and U.C. Chaturvedi. Congenital infection of mice with Japanese encephalitis virus. *Infect. Immun. 34*:26–29, 1981.

Meegan, J.M., R.H. Watten, and L.W. Laughlin. Clinical experience with Rift Valley fever in humans during the 1977 Egyptian epizootic. Contrib. Epidem. *Biostat. 3*:114–123, 1981.

Milby, M.M., R.L. Nelson, and P.T. McDonald. Release of heterozygous translocated adult males for genetic control of *Culex tarsalis* at an isolated site. *Mosquito News 40*:83–90, 1980.

Nelson, R.L., and M.M. Milby. Effects of fluorescent marker dusts on *Culex tarsalis*, a factor in mark-release-recapture studies. *Proc. Calif. Mosq. & Vector Control Assoc.* 48:66–68, 1980.

Nelson, R.L. The pipe trap, an efficient method for sampling resting adult *Culex tarsalis* (Diptera: Culicidae). *J. Med. Entomol. 17*:348–351.

Newton, S.E., and L. Dalgarno. Antiviral activity released from *Aedes albopictus* cells persistently infected with Semliki Forest virus. *J. Virol. 47*:652–655, 1983.

Olivia, O.F.P., and D.L. Knudson. Molecular approaches in epidemiological studies: Genetic analyses of Central and South American arboviruses. International Symposium on Tropical Arboviruses and Hemorrhagic Fevers, Belem, Brazil, April 14–18, 1980. *Brazilian Academy of Sciences*, 1983.

Ou, J.-H., E.G. Strauss, and J.H. Strauss. Comparative studies of the 3' terminal sequences of several alphavirus RNAs. *Virology 109*:281–289, 1980.

Parsons, I.M., A.J. Della-Porta, and W.A. Snowdon. Developmental disorders of the fetus in some arthropod-borne virus infections. *Am. J. Med. Hyg. 30*:660–673, 1981.

Peters, C.J., and J.M. Meegan. Rift Valley fever. In J.H. Steele (ed.) Handbook Series in Zoonoses, Sect. B, Viral Zoonoses, Vol. I, pp. 403–420, G.W. Beran (Section Ed.), *CRC Press*, Boca Raton, 1981.

Purdy, M., J. Petre, and P. Roy. Cloning the double-stranded RNA genes of Bluetongue Virus, 1984.

Rao, C.D., K. Sugiyama, and P. Roy. Evolution of Bluetongue virus serotypes 17. *Amer. J. Trop. Med. & Hyg., 32*: 865–870, 1983.

Rao, C.D., A. Kiuchi, and P. Roy. Molecular basis of Bluetongue Virus neutralization. *J. Virol., 48*:627–632, 1983.

Reeves, W.C., and M.M. Milby. Assessment of the relative value of alternative approaches for surveillance and prediction of arboviral activity. *Proc. Calif. Mosq. & Vector Control Assoc. 48*:3–7, 1980.

Rice, C.M., and J.H. Strauss. Nucleotide sequence of the 26S mRNA of Sindbis virus and deduced sequence of the encoded virus structural proteins. *Proc. Nat. Acad. Sci. USA 78*:2062–2066, 1981.

Rice, C.M., and J.H. Strauss. Synthesis, cleavage and sequence analysis of cDNA complementary to the 26S mRNA of Sindbis virus. *J. Mol. Biol. 150*:315–340, 1981.

Rosen, L., D.A. Shroyer, and J.C. Lien. Transovarial transmission of Japanese encephalitis virus by *Culex tritaeniorhynchus* mosquitoes. *Am. J. Trop. Med. Hyg. 29*:711–712, 1980.

Rosen, L. Transmission transovarienne des arbovirus par les moustiques. *Medecine Tropicale, 41*:23–29, 1981.

Rosen, L., and D.A. Shroyer. Natural carbon dioxide sensitivity in mosquitoes caused by a hereditary virus. *Ann. Virol. (Inst. Pasteur) 132E*: 543–548, 1981.

Rosen, L. CO_2 sensitivity in mosquitoes infected with sigma, vesicular stomatitis and other rhabdoviruses. *Science 207*:989–991, 1980.

Rosen, L. Arthropods as hosts and vectors—experimental infections. *In* W. Schlesinger (ed.): *Togaviruses*. New York, NY, *Academic Press, Inc.*, pp. 229–239, 1980.

Rosen, L., D.A. Shroyer, and J.C. Lien. Transovarial transmission of Japanese encephalitis virus by *Culex tritaeniorhynchus* mosquitoes. *Am. J. Trop. Med. Hyg.* 29:711–712, 1980.

Rosen, L., D.J. Gubler, and P.H. Bennett. Epidemic polyarthritis (Ross-River) virus infection in the Cook Islands. *Am. J. Trop. Med. Hyg. 30*: 1294–1302, 1981.

Sawicki, D.L., and S.G. Sawicki. Short-lived minus-strand polymerase for Semliki Forest virus. *J. Virol. 34*:108–118, 1980.

Sawicki, D.L., S.G. Sawicki, S. Keranen, and L. Kaariainen. Specific Sindbis virus encoded function for minus-strand RNA synthesis. *J. Virology, 39*:348–358, 1981.

Sawicki, S.G., D.L. Sawicki, L. Kaariainen, and S. Keranen. A Sindbis virus mutant temperature-sensitive in the regulation of minus-strand RNA synthesis. *Virology 115*:161–172, 1981.

Scheefers-Borchel, U., H. Scheefers, J. Edwards, and D.T. Brown. Sindbis virus maturation in cultured mosquito cells is sensitive to actinomycin D. *Virology*, 292–301, 1981.

Schlesinger, R.W., V. Stollar, N. Sarver, and R. Regnery. Studies on alphavirus replication in mosquito cell cultures and whole mosquitoes. J. Vesenjak-Hirjan et al. (eds.): Arboviruses in the Mediterranean Countries, *Zbl. Bakt. Suppl* 9. Gustav Fischer Verlag, Stuttgart-New York, 1980.

Schlesinger, R.W., editor. "The Togaviruses, Biology, Structure, Replication" (21 chapters, 720 pages). *Academic Press*, New York, 1980.

Scott, T.W., S.W. Hildreth, and B.J. Beaty. The distribution and development of Eastern equine encephalitis virus in its enzootic vector. *Culseta melanura. Am. J. Trop. Med. Hyg.*, in press. 1984.

Shope, R.E. Arbovirus related encephalitis. *Yale J. Biol. & Med. 53*: 93, 1980.

Shope, R.E. Medical significance of togaviruses. p. 47. In Schlesinger, W. (ed.), *The Togaviruses Academic Press, New York*, 1980.

Shope, R.E. Opportunities and responsibilities of the reference center. *Am. J. Trop. Med. & Hyg.*, *30*:509–515, 1981.

Shope, R.E., G.H. Tignor, E.J. Rozhon, and D.H.L. Bishop. The association of the bunyavirus middle-sized RNA segment with mouse pathogenicity. *In* "The Replication of Negative Strand Viruses," D.H.L. Bishop and R.W. Compans (eds.), pp. 147–152, Elsevier North Holland, 1981.

Shope, R.E. Bunyaviruses *In* "Medical Microbiology: Principles and Concepts," S. Baron (ed.), pp. 1132–1142, Addison-Wesley, Menlo Park, California, 1982.

Shope, R.E. Arbovirus encephalitis. Progress in recognition and control. *In: Medical Virology*. L.M. de la Maza and E.M. Peterson (editors), pp. 147–168, Elsevier, New York, 1983.

Shroyer, D.A. and G.B. Craig, Jr. Egg hatchability and diapause in *Aedes triseriatus* (Diptera: Culicidae), temperature- and photoperiod-induced latencies. *Ann. Entomol. Soc. Am. 73*:39–43, 1980.

Smith, A. and C.R. Anderson. Turtles as potential overwintering hosts of eastern equine encephalitis virus. *J. Wildlife Dis. 16*:615-617, 1981.

Squire, K.R.E., R.Y. Chuang, B.I. Osburn, D.L. Knudson, and R.H. Doi. Rapid methods for comparing the double stranded RNA genome profiles of Bluetongue virus. *Vet. Microbiol. 8*:543–553, 1983.

Stollar, V., and F. Malinoski. The effects of adenosine and guanosine on the replication of Sindbis and vesicular stomatitis viruses in *Aedes albopictus* cells. *Virology 115*:57–66, 1981

Strauss, E.G., and J.H. Strauss. Mutants of alphaviruses: Genetics and physiology. In: The Togaviruses, R.W. Schlesinger, ed., *Academic Press*, New York, pp. 393–426, 1980

Strauss, E.G., E.M. Lenches, and M.A. Stamreich-Martin. Growth and release of several alphaviruses in chick and BHK cells. *J. Gen. Virol. 49*:297–307, 1980.

Sugiyama, K., D.H.L. Bishop, and P. Roy. Analyses of the genomes of Bluetongue viruses recovered in the United States. I. Oligonucleotide fingerprint studies that indicate the existence of naturally occurring reassortment BTV isolates. *Virology, 114*:210–217, 1981.

Sugiyama, K., D.H.L. Bishop, and P. Roy. Analyses of the genomes of Bluetongue virus isolates recovered from different states of the United States and at different times. *Amer. J. Epid.*, *115*:332–347, 1982.

Tesh, R.B. Arboviruses and their diseases—Central Asia and the Soviet Union. In: pediatric infectious diseases. R.D. Feigin and J.D. Cherry, editors. *W. B. Saunders Co.*, Philadelphia, p. 1124–1131, 1981.

Tesh, R.B. Arthritides caused by mosquito-borne viruses. *Ann Rev.-Med. 33*:31–40, 1982.

Tesh, R.B. Vertical transmission of arthropod-borne viruses of vertebrates. In: vectors of disease agents. J.J. McKelvey, B.F. Eldridge, and R.F. Harwood, editors. *Praeger Publishers*, New York. pp. 112–137, 1981.

Tesh, R.B., R.G. McLean, D.A. Shroyer, C.H. Calisher, and L. Rosen. Ross River virus infection (epidemic polyarthritis) in American Samoa. *Tr. Roy. Soc. Trop. Med. Hyg. 75*:426–431, 1981.

Tesh, R.B. Undifferentiated arboviral fevers. *In: Tropical and Geographical Medicine*. K.S. Warren and A.A.F. Mahmoud (editors). McGraw Hill Book Co., New York, pp. 660–666, 1984.

Tesh, R.B., and B.J. Beaty. Localization of California serogroup viruses in mosquitoes. In: *Progress in Clinical and Biological Research* edited by C.H. Calisher and W.H. Thompson, *vol. 123*:67–75, 1983.

Tesh, R.B., and G. Modi. Growth and transovarial transmission of Chandipura virus (Rhabdovirus: *Vesiculovirus*) in *Phlebotomus papatasi. Amer. J. Trop. Med. Hyg. 32*:621–623, 1983.

Tesh, R.B., R.G. McLean, D.A. Shroyer, C.H. Calisher, and L. Rosen. Ross River virus (Togaviridae: Alphavirus) infection (epidemic polyarthritis) in American Samoa. *Tr. Roy. Soc. Trop. Med. Hyg.* *75*:426–431, 1981.

Tidwell, M.A., D.M. Forsythe, R.L. Parker, and A.J. Main. Eastern equine encephalomyelitis virus activity in South Carolina. *J. Agr. Entom.* *1*:43–52, 1984.

Tignor, G.H., T.G. Burrage, A.L. Smith, R.E. Shope, and D.H.L. Bishop. California serogroup gene structure-function relationships: virulence and tissue tropisms. In: *California Serogroup Viruses*. C.H. Calisher and W.H. Thompson (editors), pp. 129-138, Alan R. Liss, New York, 1983.

Tooker, P., and S.I.T. Kennedy. Semliki Forest Virus multiplication in clones of *Aedes albopictus* cells. *J. Virol.* *37*:589, 1981.

Travassos da Rosa, A.P.A., R.B. Tesh, F.P. Pinheiro, J.F. Travassos, P.H. Peralta, and D.L. Knudson. Characterization of the Changuinolavirus serogroup (Reoviridae: *Orbivirus*). *Intervirology* *21*:38–49, 1984.

Travassos da Rosa, A.P.A., R.B. Tesh, F.P. Pinheiro, J.F.S. Travassos da Rosa, and N.E. Peterson. Characterization of eight new phlebotomus fever serogroup arboviruses (Bunyaviridae: *Phlebovirus*) from the Amazon Region of Brazil. *Amer. J. Trop. Med. Hyg.* *32*:1164–1171, 1983.

Turell, M.J., J.L. Hardy, and W.C. Reeves. Demonstration of transovarial transmission of California encephalitis virus in experimentally infected *Aedes melanimon*. *Proc. Mosq. & Vector Control Assoc.* *48*:15-16, 1980.

Turell, M.J., and J.L. Hardy. Carbon dioxide sensitivity of mosquitoes infected with California encephalitis virus. *Science* *209*:1029–1030.

Westaway, E.G., R.W. Schlesinger, J.M. Dalrymple, and D.W. Trent. Nomenclature of Flavivirus-Specified Proteins. *Intervirology* *14*:114–117, 1980.

Zalom, F.G., S.M. Asman, and R.P. Meyer. Determining the mating ability of irradiated *Culex tarsalis* (Diptera: Culicidae). *J. Med. Entomol.* *18*:167–169, 1981.

Zalom, F.G., S.M. Asman, and J.E. Fields. Tests for multiple insemination of females involving irradiated and unirradiated male *Culex tarsalis* (Diptera: Culicidae). *Mosquito News* *41*:154-156, 1981.

Viral Gastroenteritis

Anderson, A.O., A. Plotner, and D.H. Rubin. Effect of enteric priming with reovirus and lipoidal amine adjuvant on mucosal lymphatic tissue and antiviral IgA secretion. *Ann. NY Acad. Sci.* (in press).

Estes, M.K., D.Y. Graham, R.F. Ramig, and B.L. Ericson. Heterogeneity in the structural glycoprotein (VP7) of simian rotavirus SA11. *Virology* *122*:8–14, 1982.

Flores, J., E. Boeggeman, R.H. Purcell, M. Sereno, I. Perez, L. White, R.G. Wyatt, R.M. Chanock, and A.Z. Kapikian. A dot hybridization assay for detection of rotavirus. *Lancet* *1*:816–821, 1983.

Gebhard, R.L., H.B. Greenberg, N. Singh, P. Henry, H. Sharp, L. Kaplan, and A.Z. Kapikian. Acute viral enteritis and exacerbation of inflammatory bowel disease. *Gastroenterology*, *83*:1207–1209, 1982.

Greenberg, H.B., J. Myslinski, A.R. Kalica, R.G. Wyatt, A.Z. Kapikian, and R.M. Chanock. Use of transcription probes for genotyping rotavirus reassortants. *Virology* *121*:288–295, 1982.

Greenberg, H.B., A. Kalica, J. Flores, A. Kapikian, R. Wyatt, R. Jones, and J. Valdesuso. Gene coding assignments for rotaviruses studies with genetic reassortants and monoclonal antibodies. In R.W. Compans, et. al. (eds.): *Double-stranded RNA Viruses*, New York, *Elsevier*, 1983, pp. 289–302.

Greenberg, H.B., V. McAuliffe, J. Valdesuso, R. Wyatt, J. Flores, A. Kalica, Y. Hoshino, and N. Singh. Serologic analysis of the sub-group protein of rotavirus using monoclonal antibodies. *Infec. Immun.* *39*:91–99, 1983.

Greenberg, H.B., J. Valdesuso, K. van Wyke, K. Midthun, M. Walsh, V. McAuliffe, R.G. Wyatt, A.R. Kalica, J. Flores, and Y. Hoshino. Production and preliminary characterization of monoclonal antibodies directed at two surface proteins of rhesus rotavirus. *J. Virol.* *47*:267–275, 1983.

Hoshino, Y., R.G. Wyatt, H.B. Greenberg, A.R. Kalica, J. Flores, and A.Z. Kapikian. Isolation and characterization of an equine rotavirus. *J. Clin. Microbiol.* *18*:585–591, 1983.

Hoshino, Y., R.G. Wyatt, H.B. Greenberg, A.R. Kalica, J. Flores, and A.Z. Kapikian. Isolation, propagation and characterization of a second equine rotavirus serotype. *Infec. Immun.* *41*:1031–1037, 1983.

Hoshino, Y., R.G. Wyatt, R.G. Greenberg, A.R. Kalica, J. Flores, and A.Z. Kapikian. Serologic comparison of canine rotavirus with various simian and human rotaviruses by plague reduction neutralization and hemagglutination-inhibition tests. *Ibid.* 169–173, 1983.

Kalica, A.R., J. Flores, and H.B. Greenberg. Identification of the rotaviral gene that codes for hemagglutination and protease-enhanced plague formation. *Virology* *125*:194–205, 1983.

Kapikian, A., and R.E. Shope. Reoviruses in "Medical Microbiology: Principles and Concepts," S. Baron, (ed), pp. 1132–1142, Addison-Wesley, Menlo Park, California, 1982.

Kapikian, A.Z., R.G. Wyatt, M.M. Levine, R.E. Black, H.B. Greenberg, J. Flores, A.R. Kalica, Y. Hoshino, and R.M. Chanock. Studies in volunteers with human rotaviruses. *Dev. Biol. Stand.* *53*:209–218, 1983.

Myslinski, J., A.R. Kalica, H.B. Greenberg, R.G. Wyatt, A.Z. Kapikian, and R.M. Chanock. *In vitro* transcription of two human rotaviruses. *J. Virol.* *43*:1032–1037, 1982.

Perez, I., L. White, M. Perez, A.R. Kalica, R. Marquina, R.G. Wyatt, A.Z. Kapikian, and R.M. Chanock. Genetic relatedness among human rotaviruses as studied by RNA hybridization. *Infect. Immun.*, *37*:648-655, 1982.

Ramig, R.F. Suppression of temperature-sensitive phenotype in reovirus: an alternate pathway from ts to ts^+ phenotype. In: Animal Virus Genetics, ICN-UCLA Symposia on Molecular and Cellular Biology. Ed. by B. Fields, R. Jaenisch and C.F. Fox. *Academic Press*, New York, *Vol. 18*:633-642, 1980.

Ramig, R.F. Factors that affect genetic interaction during mixed infection with temperature-sensitive mutants of simian rotavirus SA11. *Virology 127*:91-99, 1983.

Ramig, R.F. Genetic studies with simian rotavirus SA11. In *Double-Stranded RNA Viruses*, ed. by R. Compans and D.H.L. Bishop, *Elsevier*, New York, pp. 321-327, 1983.

Ramig, R.F. Isolation and genetic characterization of temperature-sensitive mutants that define five additional recombination groups in simian rotavirus SA11. *Virology 130*: in press, 1983.

Ramig, R.F., and B.L. Petrie. Characterization of temperature-sensitive mutants of simian rotavirus SA11: protein synthesis and morphogenesis. *J. Virol.* in press, 1984.

Ramig, R.F. Principles of animal virus genetics. In *Human Viral Diseases*, ed. by B.N. Fields, J.L. Melnick, R. Chanock, R.E. Shope and B. Boizman. *Raven Press*, N.Y. in press, 1983.

Rubin, D.H., A.O. Anderson, D. Lucis, and S.M. Michalek. Potentiation of the secretory IgA response by oral and enteric administration of CP20, 961. *Ann. NY Acad. Sci* (in press).

Sereno, M., C.-J. Lai, E. Boeggeman, I. Perez, R. Purcell, A. Kalica, H. Greenberg, R. Wyatt, J. Hansen, A. Kapikian, and R. Chanock. Use of single stranded rotavirus RNA transcripts for the diagnosis of rotavirus infection: the study of genetic diversity among rotaviruses, and the molecular cloning of rotavirus genes. In R.W. Compans, et al. (eds.): *Double-stranded RNA Viruses.* New York, *Elsevier.* 1983, pp. 115-128.

Singh, N., M. Sereno, J. Flores, and A.Z. Kapikian. Monoclonal antibodies to sub-group I rotavirus. *Infect. Immun.* *42*:835-837, 1983.

Smith, A.L., D.L. Knudson, J.F. Sheridan, and F.X. Paturzo. Detection of antibody to epizootic diarrhea of infant mice (EDIM) virus. *Lab. An. Sci.* *33*:442-445, 1983.

VanKirk, D.H., R. Dolin, H.B. Greenberg, and R.M. Chanock. Oral administration of human rotavirus to volunteers: induction of illness and correlates of resistance. *J. Infec. Dis.* *147*:95-106, 1983.

Wyatt, R.G., and W.D. James. Methods of gastroenteritis virus culture *in vivo* and *in vitro*. In D.A.J. Tyrrell, et al. (eds.) *Virus Infections of the Gastrointestinal Tract.* New York, *Marcel Dekker*, pp. 13-35, 1982.

Wyatt, R.G., W.D. James, A.L. Pitman, Y. Hoshino, H.B. Greenberg, A.R. Kalica, J. Flores, and A.Z. Kapikian. Direct isolation in cell culture of human rotaviruses and their characterization into four serotypes. *J. Clin. Microbiol.* *18*:310-317, 1983.

Wyatt, R.G., A.Z. Kapikian, and C.A. Mebus. Induction in calves of crossreactive serum neutralizing antibody to human rotavirus following in utero administration of bovine rotavirus. *Ibid.* 505-508, 1983.

Rabies

Anilionis, A., W.H. Wunner, and P.J. Curtis. Structure of the glycoprotein gene in rabies virus. *Nature 294*:275-278, 1981.

Charlton, K.M., G.A. Casey, D.W. Boucher, and T.J. Wiktor. Antigenic variants in rabies virus. *Comp. Immun. Microbiol. Infect. Dis.* *5*:113-115, 1982.

Crick, J., G.H. Tignor, and K. Moreno. A new isolate of Lagos bat virus from the Republic of South Africa. *Trans. Roy. Soc. Trop. Med. Hyg.*, *76*: No. 2, 1982.

Curtis, P.J., A. Anilionis, and W.H. Wunner. Cloning of full length cDNA from the rabies virus glycoprotein gene. In: The Replication of Negative Strand Viruses (D.H.L. Bishop, R.W. Compans, eds.) Elsevier North Holland, Inc., pp. 721-725, 1981.

Dietzschold, B. Structural differences in the glycoprotein of rabies virus strains. In: The Replication of Negative Strand Viruses (D.H.L. Bishop, R.W. Compans, ed.) Elsevier North Holland, Inc., pp. 631-638, 1981.

Dietzschold, B., T.J. Wiktor, R. Macfarlan, and A. Varrichio. Antigenic structure of rabies virus glycoprotein: ordering and immunological characterization of the large CNBr cleavage fragments. *J. Virol.* *44*:595-602, 1982.

Dietzschold, B., T.J. Wiktor, W.H. Wunner, and A. Varrichio. Chemical and immunological analysis of the rabies-soluble glycoprotein. *Virology 124*:330-337, 1983.

Dietzschold, B., W.H. Wunner, T.J. Wiktor, A.D. Lopes, M. Lafon, C. Smith, and H. Koprowski. Characterization of an antigenic determinant of the glycoprotein which correlates with pathogenicity of rabies virus. *Proc. Natl. Acad. Sci. USA*, *80*:70-74, 1983.

Dietzschold, B., W.H. Wunner, M. Lafon, C.L. Smith, and A. Varrichio. Variation in glycosylation pattern of G proteins among antigenic variants of the CVS strain of rabies virus. *In* "Nonsegmented Negative Strand Viruses," D.H.L. Bishop and R.W. Compans, eds. *Academic Press*, in press, 1984.

Dietzschold, B., W.H. Wunner, R.I. Macfarlan, T.J. Wiktor, M. Kiel, R. Houghton, R.A. Larner, J.G. Sutcliffe, and H. Koprowski. The antigenic structure of the rabies virus glycoprotein. *In*: "Rabies in the Tropics," E. Kuwert, ed. *Springer-Verlag* (Wein-New York), in press, 1983.

Esh, J.B., J.G. Cunningham, and T.J. Wiktor. Vaccine-induced rabies in four cats. *J. Am. Vet. Med. Assoc. 180*:1336–1339, 1982.

Koprowski, H. Monoclonal hybridoma antibodies in the diagnosis of rabies. In: *Immunological Analysis: Recent Progresses in Diagnostic Laboratory Immunology. Masson Publishing Inc.*, New York, pp. 87–90, 1982.

Lafon, M., T.J. Wiktor, and R.I. Macfarlan. Antigenic sites of the CVS rabies virus glycoprotein. Analysis with monoclonal antibodies. *J. Gen. Virol. 64*:843–851, 1983.

Lafon, M., J. Ideler, and W.H. Wunner. Investigation of the antigenic structure of rabies virus glycoprotein by monoclonal antibodies. *In*: "Developments of Biological Standardization—Joint WHO/ABS Symposium on the Standardization of Rabies Virus," in press, 1983.

Lentz, T.L., T.H. Burrage, A.L. Smith, J. Crick, G.H. Tignor. Is the acetylcholine receptor a rabies virus receptor? *Science 215*:182–184, 1982.

Merzt, G.J., K.E. Nelson, V. Vithayasai, S. Makornkawkeyoon, E.I. Rosanoff, H. Tint, and T.J. Wiktor. Antibody responses to human diploid cell vaccine for rabies with and without human rabies immune globulin. *J. Infec. Dis. 145*, 720–727, 1982.

Minato, N., L. Reid, A. Neighbour, B.R. Bloom, and J. Holland. Interferon, NK cells, and persistent virus infection. *Ann. N.Y. Acad. Sci. 350*:42–52, 1980.

Postic, B., and T.J. Wiktor. Rabies. In: "Medical Microbiology and Infectious Diseases," W.B. Saunders Co., Philadelphia, pp. 1303–1309, 1981.

Postic, B., and T.J. Wiktor. Rhabdovirus. In: "Medical Microbiology and Infectious Disease," W.B. Saunders Co., Philadelphia, pp. 616–619, 1981.

Reagan, K.J., W.H. Wunner, T.J. Wiktor, and H. Koprowski. Anti-idiotype antibodies induce neutralizing antibodies to rabies virus. *J. Virol. 48*:660–666, 1983.

Reagan, K.J., W.H. Wunner. Early interactions of rabies virus with cell surface receptors. *In*: "Nonsegmented Negative Strand Viruses," D.H.L. Bishop and R.W. Compans, eds. *Academic Press*, in press, 1983.

Sureau, P., P. Rollin, and T.J. Wiktor. Epidemiologic analysis of antigenic variations of street rabies virus: Detection by monoclonal antibodies. *Am. J. Epidemiol. 117*:605–609, 1983.

Sureau, P., G.H. Tignor, and A.L. Smith. Antigenic characterization of the Bangai strain (ANCB-672d) of Lagos bat virus. *Ann. Virol.* (Inst. Pasteur) *131E*:25–32, 1980.

Tesh, R.B., A.P.A. Travassos, and J.S. Travassos. Antigenic relationship among rhabdoviruses infecting terrestrial vertebrates. *J. Gen. Virol. 64*:169–176, 1983.

Watson, H.D., G.H. Tignor, and A.L. Smith. Entry of rabies virus into the peripheral nerves of mice. *J. Gen. Virol. 56*:371–382, 1981.

Wiktor, T.J. Antigenic variations of rabies virus. In: The Replication of Negative Strand Viruses (D.H.L. Bishop, R.W. Compans, eds.) *Elsevier North Holland, Inc.*, pp. 943–945, 1981.

Wiktor, T.J. Les Variations Antigeniques du Virus Rabique. *Etudes parlet Anticorps Monoclonaux* Rev. Inst. Pasteur, Lyon, *14*:367–376, 1981.

Wiktor, T.J. Introduction a'l etude des variants antigeniques du virus rabique. *Comp. Immun. Microbiol. Infect. Dis. 5*, 93–94, 1982.

Wiktor, T.J., M. Lafon, B. Dietzschold, and W.H. Wunner. Recognition of antigenic variants of rabies virus by monoclonal antibodies. *In*: "Advances in Gene Technology: Molecular Genetics of Plants and Animals," W.J. Whalen and J. Schultz, eds. *Academic Press* (Miami Winter Symposium), in press, 1983.

Wunner, W.H., B. Dietzschold, P.J. Curtis, and T.J. Wiktor. Rabies subunit vaccines. *In*: "Advances in Gene Technology: Molecular Genetics of Plants and Animals," W.J. Whelan and J. Schultz, eds. *Academic Press* (Miami Winter Symposium), in press, 1983.

Wunner, W.H., B. Dietzschold, P.J. Curtis, and T.J. Wiktor. Rabies subunit vaccines. A review. *J. Gen. Virol. 64*:1649–1656, 1983.

Wunner, W.H., C.L. Smith, M. Lafon, J. Ideler, and T.J. Wiktor. Comparative nucleotide sequence analysis of the glycoprotein gene of antigenically altered rabies viruses. *In*: "Nonsegmented Negative Strand Viruses," D.H.L. Bishop and R.W. Compans, eds. *Academic Press*, in press, 1984.

Wunner, W.H. Is the acetylcholine receptor a rabies virus receptor? Trends in Neurosciences. *Elsevier Biomedical Press*, in press, 1983.

Joint Scientific Conferences

VIRAL DISEASES PANELS

Date: November 17-19, 1980

Place: Oiso, Japan

Subject: The Japanese Viral Disease Panel hosted the 14th Joint Working Conference on Viral Diseases on November 17-19, 1980 at the seaside Oiso Prince Hotel in Oiso. The first day of the scientific program included virus-vector relationships of arboviruses, status of dengue vaccines, characterization of dengue virus types by nucleic acid fingerprinting, and immune responses to a Japanese encephalitis vaccine in children. The arbovirus session ended with a round table discussion on Japanese encephalitis vaccines. Scientific presentations on the topic of viral gastroenteritis filled most of the second day. There were reports of small outbreaks of gastroenteritis in schoolchildren and adults with characterization of virus particles isolated from these outbreaks. The new technology of viral RNA reassortment was reported to be successfully demonstrated with rotaviruses and recent advances in the serotyping of rotaviruses were aired. The session ended with a roundtable discussion on approaches to immunization with human rotaviruses.

Recent advances also were presented on the characterization and biology of Hantaan virus, the etiologic agent of Korean hemorrhagic fever (hemorrhagic fever with renal syndrome) and on the efficacy of antiviral drugs against Lassa fever virus. The closing scientific presentations were on rabies virus and its activity in mice. A roundtable discussion on persistent viral infections followed.

Date: November 9-11, 1981

Place: Bethesda, Maryland

Subject: The United States Viral Diseases panel hosted the 15th Joint Working Conference on Viral Diseases at the National Institutes of Health in Bethesda, Maryland on November 9-11, 1981. The first day of the conference centered on arbovirus research with 11 individual presentations. On the second day progress in rabies research was highlighted by presentations of approaches to molecular cloning of rabies virus genes, antigenic variations of rabies virus and cell mediated immune responses to rabies virus infections. Also, brief special reviews were presented on nosocomial hazards of Lassa and Ebola virus infections and on scrapies, an unconventional virus.

A most remarkable breakthrough in the successful cultivation of human rotaviruses in cell cultures was reported by Japanese investigators in the session on viral gastroenteritis. This exciting advance electrified the conference because it assured a much faster pace of research on these fastidious viruses. Further progress on serotyping of rotaviruses was reported. It is noteworthy that collaborative efforts that had been initiated between Japanese and American investigators through the USCMSP were accelerating the pace of research on rotaviruses in both countries.

Date: July 19-21, 1982

Place: Tokyo, Japan

Summary: The review of the Viral Diseases Panels by the Joint Committee was initiated as the Panels held their 16th Working Conference on rabies, arboviruses, Korean hemorrhagic fever and viral gastroenteritis in Tokyo on July 19-21, 1982. Introductory remarks acknowledged the death of Dr. William Scherer, former Chairman of the U.S. Panel. The scientific sessions began with 11 presentations on viral gastroenteritis. Considerable emphasis focused on the proteins of rotaviruses as the new technologies opened new approaches to study these viruses. A single report on the prevalence of Reye's Syndrome in Japan and reviews of Creutzfeld-Jacob disease and of Visna virus were presented as special topics. The session on rabies covered four presentations including one on a rabies vaccine of suckling mouse brain origin developed by a Japanese investigator. The final session was devoted to 13 presentations on arboviruses. Roundtable discussions followed each of the major scientific sessions.

Date: September 7-9, 1983

Place: San Diego, California

Subject: The 17th Joint Panel Meeting was hosted by the U.S. Panel at the University of California, San Diego on September 7-9, 1983 at the LaJolla, California campus. The opening session included 10 presentations on arboviruses and revealed the impact of the new molecular virological techniques on arboviral research. A session on hemorrhagic fever with renal syndrome (HFRS) revealed that viruses related to Hantaan virus are found in rodents in many areas of the world. The session on viral gastroenteritis covered 14 presentations. Again this year, serotyping of rotaviruses was a prominent topic and reemphasized the collaboration between Japanese and American investigators. The last session was on rabies and was introduced by a memorial lecture in honor of Dr. Seiichi Matsumoto, a member of the Japanese Panel and Dr. Akira Kondo, also a renowned contributor to rabies research and participant in the U.S.-Japan CMSP. Both met untimely deaths this year.

Date: September 9–11, 1984

Place: Oiso, Japan

Subject: The 18th Joint Panel Meeting on Viral Diseases was held in Oiso, Japan on September 9–11, 1984. The opening session on viral gastroenteritis comprised 11 presentations which engendered lively discussions by many of the participants. The advances in rotavirus research toward development of rotavirus vaccines were remarkable. The session on rabies included four presentations followed by a provocative roundtable discussion on future directions in rabies virus research. A special lecture in the program concerned viral diseases of veterinary importance. The final session included scientific presentations on HFRS and arboviruses and was followed by a roundtable discussion on future directions in bunyavirus research. The entire working conference was characterized by spontaneous yet thoughtful discussion and interaction by participants of both countries.

IMMUNOLOGY BOARD

Drs. Yuichi Yamamura and William Paul, Chairmen respectively of the Immunology Board, U.S.-Japan Cooperative Medical Science Program

Dr. Yuichi Yamamura, Chairman (1981–)
President
Osaka University
1-1, Yamadakami, Suita-shi
Osaka 565, Japan

Dr. William E. Paul, Chairman (1981–)
Chief, Laboratory of Immunology
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, Maryland 20205

PANEL MEMBERS

Japan

Dr. Kyoichi Kanoh (1983–)
Professor
Department of Immunology
Institute of Medical Science
Tokyo University
4-6-1, Shiroganedai, Minato-ku
Tokyo 108, Japan

Dr. Tadamitsu Kishimoto (1981–)
Professor
Department of Cellular Immunology
Institute for Molecular and Cellular Biology
Osaka University
1-3, Yamadagaoka, Suita-shi
Osaka 565, Japan

Dr. Kaoru Onoe (1981–)
Professor
Department of Biochemistry
Faculty of Medicine
Kumamoto University
2-2-1, Honjo, Kumamoto-shi
Kumamoto 860, Japan

United States

Dr. Max D. Cooper (1981–)
Professor and Director
Cellular Immunobiology Unit
Department of Pediatrics and
  Microbiology
University of Alabama
Birmingham, Alabama 35294

Dr. Kimishige Ishizaka (1981–)
O'Neill Professor of Medicine and Microbiology
The Johns Hopkins University
The Good Samaritan Hospital
Baltimore, Maryland 21239

Dr. David W. Talmage (1981–)
Associate Dean for
  Research Affairs
University of Colorado Medical Center
Denver, Colorado 80262

Dr. Tomio Tada (1981-)
Professor
Department of Immunology
Faculty of Medicine
Tokyo University
7-3-1, Hongo, Bunkyo-ku
Tokyo 113, Japan

Dr. Jonathan W. Uhr (1981-)
Professor and Chairman
Department of Microbiology
University of Texas
Health Science Center at Dallas
Dallas, Texas 75235

Former Members

Dr. Tyoku Matsuhashi (1981-1983)
Director
Department of Applied Immunology
National Institute of Health
2-10-35, Kamai-Osaki, Shinagawa-ku
Tokyo 141, Japan

Japanese Immunology Board

BROAD GOALS

Immunology has made rapid advances in the last decade. The recent application of the techniques of somatic cell hybridization, molecular genetics, and long-term culture of immunocompetent cells to the study of the immune system had led to an unprecedented increase in our understanding of this field. Such progress in fundamental immunology has the potential to revolutionize the diagnosis, treatment, and prevention of immunologic and infectious diseases, including those being studied under the auspices of the U.S.-Japan Cooperative Medical Science Program. Long-term objectives of the Immunology Board are to elucidate the cellular and molecular mechanisms of the immune response and to define effective approaches for the manipulation of the immune system.

To achieve these objectives, the Board will focus its concern on the following research areas:

1. Molecular studies of the genetic organization of the lymphoid system.
2. Cellular and molecular studies of lymphocyte activation, proliferation, and differentiation.
3. Immunogenetic approaches to the study of lymphocyte interactions, with emphasis on the role of histocompatibility gene products in the regulation of the immune response.
4. Cellular and molecular studies of the regulation of the immune response, with emphasis on diagnosis, therapy, and prevention of diseases involving disordered immune function or affected by the immune response.
5. Application of techniques for the development of stable cell lines and of somatic cell hybrids to the study of the regulation of lymphocyte activation and function and to the preparation of monoclonal antibodies, lymphocyte receptors, and both specific and non-specific lymphocyte products.
6. Mechanisms to support the interaction of immunologists and the exchange of information and material between the two countries.

PROGRESS IN PRIORITY AREAS

Extensive studies in the research areas described above have contributed much new information.

Studies of the Genes Involved in the Immune System

Identification of the gene coding for the membrane domain of IgG molecules

Analysis of the genes for IgG1, IgG2a, and IgGb revealed that the gene coding for IgG consisted of 4 exons and 2 membrane exons. In the membrane domain, there is an extremely hydrophobic amino acid sequence which may be buried in lipid bilayers. The structure of this membrane domain was shown to be conserved between several immunoglobulin isotypes, suggesting its interaction with a certain common protein molecule.

Structural analysis of human epsilon gene and identification of pseudogenes

Three epsilon genes, including two pseudogenes, were identified. The first pseudogene deleted 5' portion of epsilon structural gene, and the second pseudogene was the processed gene which had completely lost introns. The LTR sequence was detected in the 5' and 3' ends of this pseudogene, suggesting that mRNA of epsilon gene was transcribed into DNA by reverse transcriptase and integrated into the host genome.

Molecular mechanism of the commitment of antigen-specificity at pre-B cell stage

Pre-B cell lines, which were able to differentiate from mu$^-$ to mu$^+$ stage during *in vitro* culture, were established by Abelson leukemia virus. By the analysis of the immunoglobulin genes of these pre-B cell lines, it was shown that the commitment of the specificity occurred by two-step DNA rearrangements, such as from DJ joining to functional VDJ formation.

Molecular cloning and expression of the gene coding for IL-2 receptor

Interleukin-2 (IL-2) receptor protein has been purified from membrane fractions of a HTLV (human T cell leukemia virus) transformed T cell line by affinity chromotography with anti-Tac antibody. Partial amino acid sequence has been determined and an IL-2 receptor gene has been cloned. As the genes for IL-2 and IL-2 receptor have been cloned, it is now possible to analyze T cell responses at the molecular level.

Studies on the Activation, Proliferation, and Differentiation of Lymphocytes

It has been demonstrated that the process of differentiation of B cells into immunoglobulin secreting cells can be dissected into three steps, i.e., activation, proliferation, and differentiation. Involvement of B cell specific growth and differentiation factors in the processes of proliferation and differentiation and the presence of two different kinds of B cell specific growth factors have been demonstrated. Human B cell specific differentiation factor has been purified to an apparent homogeneity by using an HTLV-transformed T cell line which can secrete a relatively large amount of B cell differentiation factor. In a murine system, a T hybrid cell line has been established which secretes B cell specific differentiation factor. Involvement of B cell specific growth factor and two B cell specific differentiation factors in the induction of Ig secretion in murine B cells has been demonstrated. A strain, DBA/2Ha, showed a low responsiveness to B cell specific differentiation factor(s); this low responsiveness was shown to be linked with a recessive gene on the X chromosome and to be due to insensitivity of the receptor molecules to B cell specific differentiation factor(s). By employing this defective strain, a monoclonal antibody that recognizes receptor molecules has been prepared permitting receptor molecules to be highly purified. SDS-PAGE analysis showed that receptor molecules were dimers consisting of two subunits of 60 k daltons.

Immunogenetic Studies of Lymphocyte Interactions

Immune responses are regulated by interactions between lymphocytes with distinct functions. Mutual recognition (or self recognition) of lymphocytes is required for the activation of the immune circuits. In order to analyze the molecules involved in self recognition, several helper, or suppressor T hybridomas that are involved in MHC (Major Histocompatibility Complex)-restricted immune regulations have been established. Monoclonal antibodies that react specifically with helper or suppressor T hybridomas have been prepared. By employing these hybridomas and monoclonal antibodies, molecules responsible for self recognition were identified. These molecules were not the products of MHC, but involved in the recognition of self-MHC molecules.

Genetic control of human immune responses was studied by employing cedar pollen and cell wall antigens of *staphylococci* and *Schistosoma japonicum*. The presence of an HLA-linked immune suppressor gene has been suggested. This immune suppressor gene exerts its effect by inducing suppressor T cells.

ACHIEVEMENT OF IMMUNOLOGY BOARD DURING THESE 4 YEARS

Four years have passed since establishment of the Immunology Board. During these years, immunology experienced a revolutionary change as a result of the introduction of new procedures, such as recombinant DNA and hybridoma technologies. The isolation and gene cloning of important molecules involved in the immune system have contributed to the molecular analysis of the immune response. Such advances in basic immunology are now being applied to the diagnosis and treatment of several immune disorders, infectious diseases, and malignancies.

The preparation by the application of cell hybridization of several monoclonal antibodies that recognize lymphocyte subsets or cancer cells has provided powerful tools for the diagnosis of immune disorders and cancer. In this cooperative study, a monoclonal antibody against IL-2 receptor facilitated the isolation and molecular cloning of the IL-2 receptor gene.

Recombinant DNA technology has made it possible to clone the genes for several immunoregulatory molecules, such as IL-2 and gamma-IFN, and their receptors on target cells and to understand the regulatory mechanisms of lymphocyte activation at the molecular level. These and other achievements in molecular immunology will be applied to the diagnosis and treatment of several immune disorders, infectious diseases, and cancer in the near future.

As described, immunology has contributed new information that can now be applied to the diagnosis and treatment of the diseases of particular interest to the U.S.-Japan Cooperative Medical Science Program. In addition, the studies supported by the U.S.-Japan Immunology Board have played an essential role in immunology in Japan. The immunology course held in 1983 at Susono City under the auspices of this Board contributed very much to the the training of young Japanese immunologists.

FUTURE GOALS

Molecules that regulate the immune system will be obtained one after another by the application of recombinant DNA technology. By employing such homogeneous molecules, their role in the immune system will be elucidated and improved diagnosis and treatment of diseases caused by immune disorders made possible.

PROJECTS SUPPORTED BY THE PROGRAM

1. Organization and rearrangement of immunoglobulin genes.
2. Molecular cloning and expression of IL-2 receptor genes.
3. Cellular and molecular studies of B cell growth and differentiation.
4. Purification and immunological characterization of B cell differentiation factor.
5. Isolation and characterization of macrophage-derived lymphocyte activating factors.
6. Immunological and molecular characterization of antigen-specific suppressor factor.
7. Studies on the circuits of immune suppression.
8. Studies on the human immune suppression genes.
9. Immunological studies on autoimmune diseases.

BIBLIOGRAPHY

Kishimoto, T., K. Sugimura, M. Suemura, O. Shiho, K. Maeda, Y. Yamamura, and I. Bottcher. A T15-idiotype positive, IgE class-specific suppressor T cell line and characterization of the suppressor factor. *Int. Archs. Allergy Appl. Immun.*, 66:31–38, 1981.

Kishimoto, T., K. Yoshizaki, T. Kuritani, A. Muraguchi, Y. Miki, T. Nakagawa, and Y. Yamamura. Induction of proliferation and Ig-secretion in human monoclonal B cells with anti-immunoglobulin and T cell products. In: *B lymphocytes in the Immune Response*, edt. by Klinman, Mosier, Scher, and Vitetta, 515–522, 1981.

Kikutani, H., T. Kishimoto, N. Sakaguchi, Y. Nishizawa, P. Ralph, and Y. Yamamura. Activation of cyclic AMP-dependent protein kinase activity during LPS stimulation of macrophage tumor cell line, J774.1 *Int. J. Immunopharmacology*, *3*:57–66, 1981.

Nishida, Y., T. Kataoka, N. Ishida, S. Nakai, T. Kishimoto, I. Bottcher, and T. Honjo. Cloning of mouse immunoglobulin ε gene and its location within the heavy chain gene cluster. *Proc. Natl. Acad. Sci., USA*, *78*:1581–1583, 1981.

Sugimura, K., T. Kishimoto, K. Maeda, and Y. Yamamura. Demonstration of T15-idiotype positive effector and suppressor T cells for phosphorylcholine-specific delayed type hypersensitivity (DTH) response in CBA/N or (CBA/N x BALB/c) F_1 male mice. *Eur. J. Immunol.*, *11*:455–461, 1981.

Suemura, M., O. Shiho, H. Deguchi, Y. Yamamura, I. Bottcher, and T. Kishimoto. Characterization and isolation of IgE class-specific suppressor factor (IgE-TsF). I. The presence of the binding site(s) for IgE and of the H-2 gene products in IgE-TsF. *J. Immunol.*, *127*:465–471, 1981.

Muraguchi, A., T. Kishimoto, Y. Miki, T. Kuritani, T. Kaieda, K. Yoshizaki, and Y. Yamamura. T cell replacing factor (TRF)-induced IgG-secretion in a human B blastoid cell line and demonstration of acceptors for TRF. *J. Immunol.*, *127*:412–416, 1981.

Shigemoto, S., T. Kishimoto, and Y. Yamamura. Characterization of phosphorylcholine (PC)-specific IgE-B cells in CBA/N or (CBA/N x BALB/c)F_1 male mice. *J. Immunol.*, *127*:1070–1075, 1981.

Okada, M., N. Yoshimura, T. Kaieda, Y. Yamamura, and T. Kishimoto. Establishment and characterization of human T hybrid cells secreting immunoregulatory molecules. *Proc. Natl. Acad. Sci., USA* *78*:7717–7721, 1981.

Ralph, P. and T. Kishimoto. Tumor promoter phorbol myristic acetate stimulates immunoglobulin secretion correlated with growth cessation in human B lymphocyte cell lines. *J. Clin. Invest.*, *68*:1093–1096, 1981.

Iribe, H., T. Koga, K. Onoue, S. Kotani, S. Kusumoto, and T. Shiba. Macrophage-stimulating effect of a synthetic muramyl dipeptide and its adjuvant-active and -inactive analogs for the production of T-cell activating monokines. *Cell. Immunol.*, *64*:73–83, 1981.

Matuhasi, T., M. Usui, S. Mitani, A. Yamamoto, S. Sadahiro, and H. Ikegami. A new approach to make toxoid eliciting good IgM and/or IgG but little IgE antibody responses. *Naturwissenschaften*, *68*:49, 1981.

Kakiuchi, T., H. Nariuchi, and T. Matuhasi. Fc receptors on IgM and IgG antibody-forming cells in an early immune response. *Cell. Immunol.*, *61*:124–133, 1981.

Yasuda, T., J. Ueno, and T. Matuhasi. Anti-glycolipid antibodies in Behcet disease. Inter. Conf. on Behcet's diseases, 1981.

Matuhasi, T. Present status of quality control and development of immunoserological tests in Japan. *Excerpta Medica*, *585*, 1981.

Nariuchi, H., and T. Kakiuchi. Responses of spleen cells from mice with X-linked B cell defect to polyclonal B cell activators, purified protein derivative of tuberculin, and dextran sulfate. *Cell. Immunol.*, *61*:375, 1981.

Kakiuchi, T., and H. Nariuchi. Responses of B cells with or without C3 receptors to polyclonal B cell activators. *J. Immunol.*, *127*:955, 1981.

Takatsu, K., K. Tanaka, A. Tominaga, and T. Hamaoka. X-linked B cell defect in $DBA/2H_a$ mice: Low responder-status of B cells. *In* B Lymphocytes in the Immune Response: Functional, Developmental and Interactive Properties, edt. by Klinman, Mosier, Scher, and Vitetta, Elsevier North Holland, Inc., 331–338, 1981.

Takatsu, K., A. Tominaga, and T. Hamaoka. X-linked recessive inheritance of a defective responsiveness to T-cell-replacing factor in $DBA/2H_a$ mice. *Immunol. Letters*, 3:137–143, 1981.

Takatsu, K., Y. Sano, S. Tomita, N. Hashimoto, and T. Hamaoka. Antibody against T cell-replacing factor acceptor site(s) augments *in vivo* primary IgM responses to suboptimal doses of heterologous erythrocytes. *Nature*, *292*:360–362, 1981.

Pure, E., P.C. Isakson, K. Takatsu, T. Hamaoka, S.L. Swain, R.W. Dutton, G. Dennert, J.W. Uhr, and E.S. Vitetta. Induction of B cell differentiation by T cell factors. I. Stimulation of IgM secretion by products of a T cell hybridoma and a T cell line. *J. Immunol.*, *127*:1953–1958, 1981.

Hamaoka, T., K. Takatsu, A. Tominaga, Y. Sano, S. Tomita, and N. Hashimoto. Anti-TRF acceptor site(s) antibody which substitutes for helper T cell activity in the triggering of B cells. *Nature* *292*:480–482, 1981.

Miyama-Inaba, M., T. Masuda, T. Inada, K. Takatsu, and T. Hamaoka. Immunological properties of Fc receptor on lym-

phocytes. 9. Functional analysis of FcRγ^{+} and FcRγ^{-} lymphocytes in IgE antibody responses. *Int. Archs. Allergy Appl. Immun.*, *66*:282–292, 1981.

Taniguchi, M., I. Takei, T. Saito, and T. Tokuhisa. Activation of an acceptor T cell hybridoma by a V_H^{+} I-J^{+} monoclonal suppressor factor. In *Immunoglobulin Idiotypes*, Academic Press, Inc., 397–406, 1981.

Taniguchi, M., I. Takei, T. Saito, M. Igarashi, K. Kurihara, and H. Tomioka. Antigen-specific and nonspecific suppressor factors derived from T cell hybridomas: Their function and molecular organization. *Int. Archs. Allergy Appl. Immun.*, *66*:204–211, 1981.

Kanno, M., S. Kobayashi, T. Tokuhisa, I. Takei, N. Shinohara, and M. Taniguchi. Monoclonal antibodies that recognize the product controlled by a gene in the I-J subregion of the mouse H-2 complex. *J. Exp. Med.*, *154*:1290–1304, 1981.

Taniguchi, M., T. Saito, I. Takei, and T. Tokuhisa. Presence of interchain disulfide bonds between two gene products that compose the secreted form of an antigen-specific suppressor factor. *J. Exp. Med.*, *153*:1672–1677, 1981.

Serjeantson, S.W., M.C. Vaidya, S.H. Chan, G.T. Juji, N.K. Mehra, S. Naik, and T. Sasazuki. Leprosy. *Proceedings of the 2nd Asia and Oceania Histocompatibility Workshop Conference*, eds. M.J. Simons and B.D. Tait, 379–401, 1981.

Sasazuki, T., N. Ohta, and M. Iuchi. Schistosomiasis. *Proceedings of the 2nd Asia and Oceania Histocompatibility Workshop Conference*, eds. M.J. Simons and B.D. Tait, 402–406, 1981.

Sone, T., K. Ogawa, and T. Sasazuki. Analysis of the DR2 molecules derived from Dw12 or Dw2 Homozygous B lymphoblastoid cell lines by 2 dimensional gel electrophoresis. *Proceedings of the 2nd Asia and Oceania Histocompatibility Workshop Conference*, eds. M.J. Simons, and B.D. Tait, 453–456, 1981.

Ohta, N., M. Muto, I. Kikuchi, M. Egami, Y.K. Nishimura, and T. Sasazuki. HLA-DR2 related HLA-D specificities, Dw2 and Dw12, in the Japanese population. *Proceedings of the 2nd Asia and Oceania Histocompatibility Workshop Conference*, edt. by M.J. Simons, B.D. Tait, 457–458, 1981.

Nishimura, Y., T. Sone, and T. Sasazuki. Confirmation of the HLA-linked immune suppression gene to streptococcal cell wall antigen (Is-SCW) in man. *Proceedings of the 2nd Asia and Oceania Histocompatibility Workshop Conference*, edt. by M.J. Simons, and B.D. Tait, 501–508, 1981.

Goto, M., K. Tanimoto, Y. Horiuchi, and T. Sasazuki. Family analysis of Werner's syndrome: A survey of 42 Japanese families with a review of the literature. *Clinical Genetics*, *19*:8–15, 1981.

Uno, H., T. Sasazuki, H. Tamai, and H. Matsumoto. Two genes, linked to HLA and Gm, control tne susceptibility to Graves' disease. *Nature*, *292*:768–770, 1981.

Sasazuki, T., N. Ohta, Y.K. Nishimura, T. Abe, C. Abe, and Y. Shiokawa. Japanese rheumatoid arthritis and HLA. In: *New Horizons in Rheumatoid Arthritis*, edt. by Y. Shiokawa, et al., *Excerpta Medica*, Amsterdam, 247, 1981.

Hiramatsu, K., S. Miyatani, M. Kim, S. Yamada, K. Okumura, and T. Tada. Unique T cell Ia antigen expressed on a hybrid cell line producing antigen-specific augmenting T cell factor. *J. Immunol.*, *127*:1118–1122, 1981.

Nikaido, T., S. Nakai, and T. Honjo. Switch region of immunoglobulin $C\mu$ gene is composed of simple tandem repetitive sequences. *Nature*, *292*:845–848, 1981.

Kishimoto, T. IgE class-specific suppressor T cells and regulation of the IgE response. In *Progress in Allergy*, *32*:265–317, 1982.

Kishimoto, T., M. Suemura, K. Sugimura, M. Okada, K. Nakanishi, and Y. Yamamura. Characterizations of T cell-derived immunoregulatory molecules from murine or human T hybridomas. In *Lymphokines*, edt. by M. Feldmann and M.H. Schreier, Academic Press New York, 129–160, 1982.

Kishimoto, T. Human neoplastic B cells—Monoclonal models of B-cell differentiation. In *B Lymphocytes Today*, edt. by J.R. Inglis, Elsevier Biomedical Press, 137, 1982.

Yoshizaki, K., T. Nakagawa, T. Kaieda, A. Muraguchi, Y. Yamamura, and T. Kishimoto. Induction of proliferation and Igs-production in human B leukemic cells by anti-immunoglobulins and T cell factors. *J. Immunol.*, *128*:1296–1301, 1982.

Miki, Y., H. Kishi, A. Muraguchi, S. Kishimoto, Y. Yamamura, and T. Kishimoto. The requirement for esterase activation in T cell replacing factor (TRF)-induced IgG production in a human B blastoid cell line. *J. Immunol.*, *128*:675–678, 1982.

Muraguchi, A., T. Tashibana, Y. Miki, T. Kuritani, H. Kishi, S. Kishimoto, T. Yamamura, and T. Kishimoto. Depressed functions of T cells and the presence of suppressor macrophages in patients with sarcoidosis. *J. Clin. Immunol. and Immunopathol.*, *23*:189–201, 1982.

Ralph, P., and T. Kishimoto. Tumor promoter phorbol myristic acetate is a T-cell dependent inducer of immunoglobulin secretion in human lymphocytes. *J. Clin. Immunol. and Immunopathol.*, *22*:340–348, 1982.

Sugimura, K., K. Nakanishi, K. Maeda, S. Kashiwamura, M. Suemura, O. Shiho, Y. Yamamura, and T. Kishimoto. The involvement of two distinct subsets of T cells for the expression of the IgE class-specific suppression. Establishment and characterization of PC-specific, antigen non-specific T hydridomas. *J. Immunol.*, *128*:1637–1644, 1982.

Sugiyama, H., S. Akira, N. Yoshida, S. Kishimoto, Y. Yamamura, P. Kincade, T. Honjo, and T. Kishimoto. Relationship between the rearrangement of immunoglobulin genes, the appearance of a B lymphocyte specific antigen, and im-

munoglobulin synthesis in murine pre-B cell lines. *J. Immunol.*, *128*:2793–2797, 1982.

Suemura, M., K. Sugimura, A. Ishizaka, S. Kobatake, O. Shiho, H. Deguchi, S. Kishimoto, Y. Yamamura, and T. Kishimoto. Regulation of the IgE response by IgE class-specific suppressor T hybridomas. In: *Current Topics in Microbiology and Immunology*, edt. by M. Cooper, et al., Springer-Verlag Berlin Heidelberg, 100:79–88, 1982.

Kaieda, T., M. Okada, N. Yoshimura, S. Kishimoto, Y. Yamamura, and T. Kishimoto. A human helper T cell clone secreting both killer helper factor(s) and T cell-replacing factor(s). *J. Immunol.*, *129*:46–51, 1982.

Kishimoto, T., Y. Miki, H. Kishi, A. Muraguchi, S. Kishimoto, and Y. Yamamura. IgG induction in a human B cell line by red cell-mediated microinjection of the cytoplasm from T cell factor-stimulated B cells. *J. Immunol.*, *129*:1367–1371, 1982.

Miki, Y., H. Kishi, A. Muraguchi, S. Maruyama, S. Kishimoto, Y. Yamamura, C.Y. Wang, and T. Kishimoto. Induction of IgG production in a human monoclonal B lymphoblastoid cell line by a B cell-specific monoclonal antibody. *J. Immunol.*, *129*:1921–1925, 1982.

Nakanishi, K., K. Sugimura, Y. Yaoita, K. Maeda, S. Kashiwamura, T. Honjo, and T. Kishimoto. A T15-idiotype positive T suppressor hybridoma does not use the T15 V_H gene segment. *Proc. Natl. Acad. Sci. USA*, *79*:6984–6988, 1982.

Kishimoto, T., M. Okada, T. Kaieda, and N. Yoshimura. Immunoregulatory molecules (lymphokines) secreted from human monoclonal T cell lines. In *Self-Defense Mechanisms — Role of Macrophage*, edt. by D. Mizuno, S.A. Cohn, K. Takeya, N. Ishida, University of Tokyo Press, 137–146, 1982.

Yoshida, N., T. Watanabe, N. Sakaguchi, H. Kikutani, S. Kishimoto, Y. Yamamura, and T. Kishimoto. Induction of 19S IgM secretion in a murine pre-B cell line, 70Z/3 by cell hybridization with non-secreting myeloma cells. *Molecular Immunology*, *19*: 1982.

Kishimoto, T., K. Yoshizaki, M. Okada, Y. Miki, T. Nakagawa, N. Yoshimura, H. Kishi, and Y. Yamamura. Activation of human monoclonal B cells with anti-Ig and T cell-derived helper factor(s) and biochemical analysis of the transmembrane signaling in B cells. In *B and T cell Tumors: Biological and Clinical Aspects*. UCLA Symposia on Molecular and Cellular Biology, Vol. XXIV., edt. by E. Vitetta and C.F. Fox, Academic Press, New York, NY., 375–389, 1982.

Iribe, H., T. Koga, and K. Onoue. Production of T cell-activating monokine of guinea pig macrophages induced by MDP and partial characterization of the monokine. *J. Immunol.*, *129*:1029–1032, 1982.

Onoue, K., T. Taniyama, H. Iribe, S. Sakamoto, and T. Koga. Monokines in T cell activation. In *Self-Defense Mechanisms: Role of Macrophages*, edt. by D. Mizuno, Z.A. Cohn, K. Takeya, N. Ishida, University of Tokyo Press, 147–158, 1982.

Teranishi, T., T. Hirano, N. Arima, and K. Onoue. Human helper T cell factor(s) (ThF). II. Induction of IgG production in B lymphoblastoid cell lines and identification of T cell-replacing factor-(TRF) like factor(s). *J. Immunol.*, *128*:1903–1908, 1982.

Naruchi, H., and T. Kakiuchi. Antibody responses to various T-independent antigens of B cells with or without C3 receptors and adherent cell requirement for these responses. *J. Immunol.*, *128*:161, 1982.

Mohri, S., T. Watanabe, and H. Naruchi. Studies of the immunological activities of the outer membrane protein from Escherichia coli. *Immunology*, *46*:271, 1982.

Minami, M., N. Honji, and M.E. Dorf. Mechanism responsible for the induction of I-J restrictions on Ts3 suppressor cells. *J. Exp. Med.*, *156*:1502, 1982.

Minami, M., S. Furusawa and M.E. Dorf. I-J restrictions on the activation and interaction of parental and FI-derived Ts3 suppressor cells. *J. Exp. Med.*, *156*:456, 1982.

Minami, M., K. Okuda, M.E. Sunday, and M.E. Dorf. H-2K-, H-2I, and H-2D-restricted hybridoma contact sensitivity effector cells. *Nature*, *5865*:351, 1982.

Iwata, M., M. Katsuta, T. Tanji, H. Tokiwa, and T. Matuhasi. Antigenicity of Beta-lactam antibiotic preparations: production of IgE antibodies to Beta-lactam antibiotic and their cross-reaction within the antibiotic group. *Int. Archs. Allergy Appl. Immun.*, *68*:35–40, 1982.

Mitani, S., A. Yamamoto, H. Ikegami, M. Usui, and T. Matuhasi. Immunoglobulin E-suppressing and immunoglobulin G-enhancing tetanus toxoid prepared by conjugation with pullulan. *Infection and Immunity*, *36(3)*: 971–976, 1982.

Arimitsu, Y., S. Kobayasi, K. Akama, and T. Matuhasi. Development of a simple serological method for diagnosing Leptospirosis: a microcapsule agglutination test. *Journal of Clinical Microbiology*, *15(5)*:835–841, 1982.

Matuhasi, T., M. Usui, and M. Iwata. IgA- and IgG-enhancing but IgE-suppressing characteristics of lipopolysaccharide- or cholesterol-ovalbumin conjugate intubated to mice. *Naturwissenschaften*, *69*:397, 1982.

Usui, M., N. Tamura, T. Matuhasi, Y. Mitsui, and Y. Iitake. The crystals of IgG Fc-like fragment by natural cleavage of human IgG during 9 years' storage. *Japan. J. Exp. Med.*, *52(3)*:149–151, 1982.

Matuhasi, T., and H. Ikegami. Elevation of levels of IgE antibody to tetanus toxin in individuals vaccinated with diphtheria-pertussis-tetanus vaccine. *Journal of Infectious Disease*, *146*:290, 1982.

Mizuguchi, J., T. Kakiuchi, H. Naruchi, and T. Matuhsi. Different effects of LPS-induced macrophage factor on antibody response to TI-1 and TI-2 antigens. *Immunology*, *47*:183–192, 1982.

Taniguchi, N., M. Usui, K. Okuda, and T. Matuhasi. Hapten-pullulan conjugate-induced CMI suppression: demonstration of a common pathway of suppressor cells involving idiotypic interactions. *J. Immunol.*, *129*:1816–1822, 1982.

Mizuguchi, J., T. Yosida, Y. Sato, S. Kondo, F. Nagaoka, and T. Matuhasi. Requirement of at least two distinct monoclonal antibodies for efficient neutralization of tetanus toxin *in vivo*. Naturwissenschaften, 69(597), 1982.

Takatsu, K., and T. Hamaoka. DBA/2Ha mice as a model of an X-linked immunodeficiency which is defective in the expression of TRF-acceptor site(s) on B lymphocytes. *Immunol. Rev.*, *64*:25–55, 1982.

Howard, M., J. Farrar, M. Hilfiker, B. Johnson, K. Takatsu, T. Hamaoka, and W.E. Paul. Identification of a T cell-derived B cell growth factor distinct from interleukin 2. *J. Exp. Med.*, *155*:914–923, 1982.

Takatsu, K., Y. Sano, N. Hashimoto, S. Tomita, and T. Hamaoka. Acceptor site(s) for T cell-replacing factor (TRF) on B lymphocytes. I. TRF-substituting activity of anti-TRF acceptor site(s) antibody in the triggering of B cells. *J. Immunol.*, *128*:2575–2580, 1982.

Tominaga, A., K. Takatsu, and T. Hamaoka. Acceptor site(s) for T cell-replacing factor (TRF) on B lymphocytes. II. Activation of B cells by cross-linkage or aggregation of the TRF acceptor molecule. *J. Immunol.*, *128*:2581–2585, 1982.

Takatsu, K., Y. Hashimoto, N. Hashimoto, Y. Sano, and T. Hamaoka. TRF-responsive B cells constitute a subpopulation distinct from the other B cell subpopulation. *In* B and T Cell Tumors, Academic Press, Inc., 161–165, 1982.

Takatsu, K., S. Tomia, Y. Hara, N. Ishii, T. Kanatani, and T. Hamaoka. Role of T cell-replacing factor (TRF) produced by monoclonal T cell hybrid in B cell differentiation. In Lymphokines, Interleukins and Cytokines, Academic Press, in press, 1982.

Tokuhisa, T., Y. Komatsu, Y. Uchida, and M. Taniguchi. Monoclonal alloantibodies specific for the constant region of T cell antigen receptors. *J. Exp. Med.*, *156*:888–897, 1982.

Yamauchi, K., M. Taniguchi, D. Green, and R.K. Gershon. The use of a monoclonal I-J-specific antibody to distinguish cells in the feedback suppression circuit from those in the contrasuppressor circuit. *Immunogenetics*, *16*:551–558, 1982.

Taniguchi, M., T. Tokuhisa, M. Kanno, and T. Honjo. An antigen-specific suppressor T cell factor controlled by two genes in the immunoglobulin heavy chain linkage group and in the I-J subregion of the H-2 complex. *In: Current Topics in Microbiology and Immunology*, edt. by M. Cooper, et. al., Springer-Verlag Berlin Heidelberg, *100*:33–41, 1982.

Taniguchi, M., T. Tokuhisa, M. Kanno, Y. Yaoita, A. Shimizu, and T. Honjo. Reconstitution of antigen-specific suppressor activity with translation products of mRNA. *Nature*, *298*:172–174, 1982.

Taniguchi, M., T. Saito, I. Takei, M. Kanno, T. Tokuhisa, and H. Tomioka. Suppressor T-cell hybridomas and their soluble products. *In: Lymphokines, Academic Press, Inc.*, *5*:77–117, 1982.

Tokuhisa, T., and M. Taniguchi. Two distinct allotypic determinants on the antigen-specific suppressor and enhancing T cell factors that are encoded by genes linked to the immunoglobulin heavy chain locus. *J. Exp. Med.*, *155*:126–139, 1982.

Isohisa, I., F. Numano, H. Maezawa, and T. Sasazuki. Hereditary factors in Takayasu's disease. *Angiology*, *33*:98, 1982.

Ohta, N., Y.K. Nishimura, M. Iuchi, and T. Sasazuki. Immunogenetic analysis of patients with post-schistosomal liver cirrhosis in man. *Clin. Exp. Immunol.*, *49*:493–499, 1982.

Ohta, N., Y.K. Nishimura, K. Tanimoto, Y. Horiuchi, C. Abe, Y. Shiokawa, T. Abe, M. Katagiri, T. Yoshiki, and T. Sasazuki. Association between HLA and Japanese patients with rheumatoid arthritis. *Human Immunology*, *5*:123–132, 1982.

Sasazuki, T., H. Uno, N. Yasuda, H. Tamai, and H. Matsumoto. Evidence for HLA-linked and Gm-linked genes in Graves' disease. *In: Human Genetics, Part B: medical Aspects*, edt. by Batsheva Bonne-Tamir, A.R. Liss, Inc., New York, 65–76, 1982.

Sasazuki, T. HLA: Genetic control of immune response and disease susceptibility. *Japanese Journal of Human Genetics*, *27*:81–94, 1982.

Sasazuki, T., Y.K. Nishimura, S. Mineshita, H. Miyashita, and G. Inaba. Genetic analysis of Behcet disease. *In: Behcet's Disease: Pathogenetic Mechanism and Clinical Future*, edt. by Inaba, G., Univ. Tokyo Press, 33–40, 1982.

Hiramatsu, K., A. Ochi, S. Miyatani, A. Segawa, and T. Tada. Monoclonal antibodies against unique I-region gene products expressed only on mature functional T cells. *Nature*, *296*:666–668, 1982.

Ochi, A., M. Nonaka, K. Hayakawa, K. Okumura, and T. Tada. Two loci in I-J subregion of the H-2 complex controlling molecules selectively expressed on suppressor and helper T cells. *J. Immunol.*, *129*:227–231, 1982.

Okumura, K., K. Hayakawa, and T. Tada. Cell-to-cell interaction controlled by immunoglobulin genes: role of Thy-1^-, Lyt-1^+, Ig$^+$ (B') cell in allotype-restricted antibody production. *J. Exp. Med.*, *156*:443–453, 1982.

Kataoka, T., T. Nikaido, T. Miyata, K. Moriwaki, and T. Honjo. The nucleotide sequences of rearranged and germline immunoglobulin V_H genes of a mouse myeloma MC101 and evolution of V_H genes in mouse. *J. Biol. Chem.*, *257*:277–285, 1982.

Nakai, S., V. Oi, L.A. Herzenberg, H. Yamagishi, and T. Honjo. Nucleotide sequences encoding membrane domains are conserved among immunoglobulin gamma subclass genes. *Biomedical Research*, *3*:37–45, 1982.

Ishida, N., S. Ueda, H. Hayashida, T. Miyata, and T. Honjo. The nucleotide sequence of the mouse immunoglobulin epsilon gene: comparison with the human epsilon gene sequence. *The EMBO Journal*, *1*:1117-1123, 1982.

Ueda, S., S. Nakai, Y. Nishida, H. Hisajima, and T. Honjo. Long terminal repeat-like elements flank a human immunoglobulin epsilon pseudogene that lacks introns. *The EMBO Journal*, *1*:1539-1544, 1982.

Yamawaki-Kataoka, Y., S. Nakai, T. Miyata, and T. Honjo. Nucleotide sequences of gene segments encoding membrane domains of immunoglobulin γ chains. *Proc. Natl. Acad. Sci., USA*, *79*:2623-2627, 1982.

Yoshizaki, K., T. Nakagawa, K. Fukunaga, T. Kaieda, S. Maruyama, S. Kishimoto, Y. Yamamura, and T. Kishimoto. Characterization of human B cell growth factor (BCGF) from a cloned T cell or mitogen-stimulated T cells. *J. Immunol.*, *130*:1241-1246, 1983.

Maruyama, S., T. Naito, H. Kakita, S. Kishimoto, Y. Yamamura, and T. Kishimoto. Preparation of a monoclonal antibody against human monocyte lineage. *J. Clin. Immunol.*, *3*:57-64, 1983.

Suemura, M., A. Ishizaka, S. Kobatake, K. Sugimura, K. Maeda, K. Nakanishi, S. Kishimoto, Y. Yamamura, and T. Kishimoto. Inhibition of IgE production in B hybridomas by IgE class-specific suppressor factor from T hybridomas. *J. Immunol.*, *130*:1056-1060, 1983.

Okada, M., N. Sakaguchi, N. Yoshimura, H. Hara, K. Shimizu, N. Yoshida, K. Yoshizaki, S. Kishimoto, Y. Yamamura, and T. Kishimoto. B cell growth factors (BCGF) and B cell differentiation factor (BCDF) from human T hybridomas: Two distinct kinds of BCGFs and their synergism in B cell proliferation. *J. Exp. Med.*, *157*:583-590, 1983.

Kishimoto, T. Human neoplastic B cells—Monoclonal models of B-cell differentiation—. *Immunology Today*, *4*:117-120, 1983.

Sugiyama, H., S. Akira, H. Kikutani, S. Kishimoto, Y. Yamamura, and T. Kishimoto. Functional V region formation during *in vitro* culture of a murine immature B precursor line. *Nature*, *303*:812-815, 1983.

Kishimoto, T., Y. Miki, H. Kishi, A. Muraguchi, K. Yoshizaki, and Y. Yamamura. T cell replacing factor (TRF)-induced IgG production in a human B cell line and the mechanism of transmembrane signaling through TRF-acceptors. *In: Protein Conformation As An Immunological Signal*, edt. by F. Celade, V.N. Schumaker and E.E. Sercarz, Plenum Publishing Corporation, 487-498, 1983.

Akira, S., H. Sugiyama, N. Yoshida, H. Kikutani, Y. Yamamura, and T. Kishimoto. Isotype switching in murine pre-B cell lines. *Cell*, *34*:545-556, 1983.

Kishi, H., Y. Miki, H. Kikutani, Y. Yamamura, and T. Kishimoto. Sequential induction of a phospholipid methylation and serine esterase activation in a B cell differentiation factor (BCDF)-stimulated human B cell line. *J. Immunol.*, *131*:1961-1965, 1983.

Kishimoto, T., H. Deguchi, M. Suemura, and Y. Yamamura. Regulation of human IgE response by IgE class-specific suppressor T cells or suppressor factor(s). *Monographs in Allergy*, *18*:75-82, 1983.

Kishimoto, T. Regulation of proliferation and differentiation of B lymphocytes by T cell-derived immunoregulatory molecules. *In: Humoral Factors In Host Defense*, edt. by Y. Yamamura, H. Hayashi, T. Honjo, T. Kishimoto, M. Muramatsu, T. Osawa, 93-109, 1983.

Kishimoto, T. Signals for B cell activation and mechanism of transmembrane signaling. *In* Progress in Immunology, edt. by Y. Yamamura, and T. Tada, Academic Press, 691-700, 1983.

Okada, M., K. Shimizu, T. Kaieda, K. Yoshizaki, and T. Kishimoto. Growth and differentiation factors for B cells from human T cell clones. *Lymphokine Research*, *2*:113-118, 1983.

Yodoi, J., M. Suemura, and T. Kishimoto. $Fc_{\epsilon}R$ and $Fc_{\gamma}R$ expressed on murine IgE-specific suppressor T hybridomas. *Microbiol. Immunol.*, *27*:877-891, 1983.

Yaoita, Y., N. Matsunami, C.Y. Choi, H. Sugiyama, T. Kishimoto, and T. Honjo. The D-J_H complex is an intermediate to the complete immunoglobulin heavy-chain V-region gene. *Nucleic Acids Research*, *11*:7303-7316, 1983.

Deguchi, H., M. Suemura, A. Ishizaka, Y. Ozaki, S. Kishimoto, Y. Yamamura, and T. Kishimoto. IgE class-specific suppressor T cells and factors in humans. *J. Immunol.*, *131*:2751, 1983.

Hirano, T., T. Sudo, T. Teranishi, K. Fujimoto, M. Iizuka, S. Kobayashi, and K. Onoue. Translation of human interleukin 2 mRNA in Xenopus laevis oocytes. *Microbiol. Immunol.*, *27*:87-94, 1983.

Maeda, S., N. Nishino, K. Obaru, S. Mita, H. Nomiyama, K. Shimada, K. Fujimoto, T. Teranishi, T. Hirano, and K. Onoue. Cloning of Interleukin 2 mRNAs from human tonsils. *Biochem. Biophys. Res. Commun.*, *in press*.

Taniyama, T., and K. Onoue. Physicochemical properties of T-cell-activating monokines in guinea pigs. *Cell. Immunol.*, *in press*.

Hirano, T., T. Teranishi, and K. Onoue. Human helper T cell factor(s). III. Characterization of B cell differentiation factor I (BCDF I). *J. Immunol.*, *in press*.

Sakamoto, S., K. Onoue, and M. Ohishi. T cell-activating monokines in guinea pigs: comparison of high and low molecular weight factors. *Microbiol. Immunol.*, *in press*.

Iwata, M., H. Tokiwa, and T. Matuhasi. Detection and characterization of polymers in cephalothin by passive cutaneous anaphylaxis in mice. *Int. Archs. Allergy Appl. Immun.*, *70*:132-137, 1983.

Kobayasi, S., S. Yamaya, T. Sugawara, and T. Matuhasi. Microcapsule agglutination test for Treponema pallidum antibodies—a new serodiagnostic test for syphilis. *Brit. J. Vener. Dis.*, *59*:1-7, 1983.

Mori, T., T. Sudo, and K. Kano. Expression of heterophil forssman antigen on cultured malignant cell lines. *J. Nat. Cancer Inst.* *70(5)*:811-817, 1983.

Takahayashi, K., N. Suzuki, M. Kanno, K. Imai, T. Tokuhisa, H. Tomioka, and M. Taniguchi. Cell cycle-dependent expression of antigen-binding and I-J-bearing molecules on suppressor T cell hybridomas. *J. Immunol.*, *130*:2552-2556, 1983.

Taniguchi, M. Functional roles of two polypeptide chains that compose an antigen-specific suppressor T cell factor. *In* Ir Genes, Past, Present, and Future, edt. by C.W. Pierce, S.E. Cullen, et. al., The Humana Press, Inc., 575-583, 1983.

Numano, F., I. Isohisa, N. Ohta, and T. Sasazuki. HLA-DR MT and MB antigens in Takayasu disease. *Tissue Antigens*, *21*:208-212, 1983.

Sasazuki, T., Y. Nishimura, M. Muto, and N. Ohta. HLA-linked genes controlling the immune response and disease susceptibility. *Immunol. Rev.*, *70*:51-75, 1983.

Nishimura, Y., and T. Sasazuki. Suppressor T cells control the HLA-linked low responsiveness to streptococcal antigen in man. *Nature*, *302*:67-69, 1983.

Ohta, N., M. Iuchi, and T. Sasazuki. Antigen specific suppressor T cell in the low responders to schistosomal antigen. *J. Immunol.*, *in press*.

Tada, T., A. Ochi, S. Miyatani, R. Abe, J. Yagi, and K. Yamauchi. Epitope organizations of I region products associated with antigen-specific augmenting and suppressor T cell factors. *In* Ir Genes, Past, Present, and Future, edt. by C.W. Pierce, S.E. Cullen, J.A. Kapp, B.D. Schwartz, and D.C. Shreffler, *The Humana Press*, 599-607, 1983.

Nakajima, P.B., A. Ochi, F.L. Owen, and T. Tada. Presence of Igt-C and I-A subregion-encoded determinants on distinct chains of monoclonal antigen-specific augmenting factor derived from a T cell hybridoma. *J. Exp. Med.*, *157*:2110-2120, 1983.

Tada, T., G. Suzuki, R. Abe., Y. Kimagai, K. Hiramatsu, and S. Miyatani. IgV_H and MHC-restricted regulatory circuit in the immune response. *In* Immunoregulation, edt. by N. Fabris, E. Geraci, J. Hadden, and N.A. Mitchison, Plenum Publishing Corporation, 1-11, 1983.

Nigone, N., J. Feder, H. Cann, B.V. West, J. Hwang, N. Takahashi, T. Honjo, A. Piazza, and L.L. Cavalli-Sforza. Multiple DNA fragment polymorphisms associated with immunoglobulin μ chain switch-like regions in man. *Proc. Natl. Acad. Sci., USA*, *80*:467-471, 1983.

Hisajima, H., Y. Nishida, S. Nakai, N. Takahashi, S. Ueda, and T. Honjo. Structure of the human immunoglobulin Cε2 gene, a truncated pseudogene: Implications for its evolutionary origin. *Proc. Natl. Acad. Sci., USA*, *80*:2995-2999, 1983.

Shimizu, A., Y. Yaoita, K. Moriwaki, K. Kondo, and T. Honjo. Japanese wild mouse, mus musculus molossinus has duplicated immunoglobulin $\gamma 2a$ genes. *Nature*, *in press*.

AVAILABLE

U.S. Immunology Board

BROAD GOALS

In 1980, the Joint Committee of the U.S.-Japan Cooperative Medical Science Program sponsored an Immunology Symposium as a first step to adding immunology to the Program. A year later, the Immunology Board was formed. Because this was a discipline, rather than a disease-oriented area, it was named a Board instead of a Panel and funded by the Ministry of Education rather than the Ministry of Health and Welfare.

In reaching the decision to add an Immunology Board, the Joint Committee noted that in the field of immunology rapid advances had been made during the last decade. The recent introduction of techniques of somatic cell hybridization, molecular genetics, and long term culture of immunocompetent cells applied to studies of the immune system has caused an unprecedented increase in our understanding of this field. Such progress in fundamental immunology has the potential to revolutionize approaches to the diagnosis, treatment, and prevention of immunologic and infectious diseases, including those conditions studied under the auspices of the U.S-Japan Cooperative Medical Science Program.

Consequently, the Joint Committee agreed that a long-term goal of the Immunology Board should be the study and elucidation of cellular and molecular mechanisms of the immune response and the definition of effective approaches to the manipulation of the immune system.

In order to achieve these goals, the Board will focus its concern on the following areas:

- Molecular studies of the genetic organization of the lymphoid system.
- Cellular and molecular studies of lymphocyte activation, proliferation and differentiation.
- Immunogenetic approaches to the study of lymphocyte interactions, with emphasis on the role of histocompatibility gene products in the regulation of the immune response.
- Cellular and molecular studies of the regulation of the immune response, with emphasis on diagnosis, therapy, and prevention of diseases involving both disordered immune function or affected by the immune response.
- Application of techniques for the development of stable cell lines and somatic cell hybrids to the study of the regulation of lymphocyte activation and function and to the preparation of monoclonal antibodies, lymphocyte receptors, specific and non-specific lymphocyte products.
- Mechanisms to support the interaction of immunologists and the exchange of information and materials between concerned workers in the two countries.

PROGRESS IN PRIORITY AREAS

During the past 3½ years, considerable progress has been made by U.S. investigators in several of these areas. This progress is, to a large extent, attributable to the shift from descriptive to analytic approaches to studies of the immune phenomena. The experimental immunologist now has the capability to visualize and isolate specific cellular components of the immune system for precise studies of the molecular mechanisms responsible for their functional activity. With appropriate monoclonal antibodies prepared by hybridoma technology, these cells can be identified and sampled using the fluorescence activated cell sorter. By suitable culture techniques, the selected cells can be cloned and propagated in large volumes to provide a sufficient supply of sub-cellular components for molecular characterization. Isolated fractions can be further defined by analytic techniques to provide detailed structural information and permit definition of the nucleotide sequence controlling the synthesis of important cellular constituents. Using this information, relevant molecules can be synthesized in sufficient quantities and purity by recombinant DNA technology to permit detailed studies of their functional role in the immune system. With such capabilities, contemporary immunologists are intensively exploring the molecular biology of the immune system in order to acquire a complete understanding of the system's normal and abnormal functions.

DIFFERENTIATION AND MATURATION OF THE IMMUNE SYSTEM

A full understanding of the ontogeny of immunocompetent cells is required for selective manipulation of the immune system and for correcting imbalances and deficiencies in host defense mechanisms. Thus, definition of the origin, differentiation pathways, and functional roles of the various subpopulations of lymphocytes are the focus of considerable investigative effort. Although it is certain that one population is thymus independent (B), its progenitors and mechanism of functional maturation are still not precisely identified. Studies of T and B cell lineages have been greatly facilitated by the recognition that certain lymphocyte surface molecules are characteristically expressed at specific stages of differentiation and maturation and by the availability of specific monoclonal antibodies to identify these marker antigens.

Much emphasis is being placed on B cell ontogeny because it has been recognized that events controlling the expression of immunoglobulin genes occur very early in B cell development, providing the opportunity to identify the genetic mechanism responsible for immunoglobulin synthesis and diversity. Also, many clinically relevant abnormalities in antibody producing cells reflect defects which occur and become evident very early in B cell differentiation. Furthermore, monoclonal antibodies that have been developed against phenotypic and functional markers which define a series of sequential steps in B cell differentiation can provide the means for separating and independently studying the characteristics and functions of cells at each developmental stage. Transformed cell lines representing many of the stages of B cell differentiation also are available and hybridoma technology has provided the opportunity to ar-rest and maintain normal B cells at varying developmental stages. It is likely that techniques for developing and maintaining clones of genetically unaltered B cells soon will be available.

Using such approaches, considerable information concerning the regulation of B cell growth and differentiation has been obtained recently. It is now generally accepted that the developmental process leading from multipotential stem cells to pre-B cells to immature B lymphocytes bearing cell surface immunoglobulin (IgM) are genetically programmed and are not antigenically driven. Nevertheless, the respective roles of self or exogeneous antigens, mitogens, or T cell factors, in contrast to intrinsic genetic programs in mediating succeeding steps in differentiation, particularly the expression of diverse immunoglobulin isotypes, are less well defined.

Analysis of the expression of isotype diversity has contributed importantly to the development of current understanding of the process of B cell differentiation. Sequential changes in expression of isotypes either in the cytoplasm or on the surface of B cells during ontogeny or after antigenic or mitogenic stimulation have served as the major phenotypic markers for discerning sequential steps in differentiation. In the recent past, the segment of mouse chromosome 12, which contains the family of genes encoding immunoglobulin heavy chains, has been analyzed in considerable depth and detail. The resulting information concerning the organization and structure of immunoglobulin genes in their germ line configuration and the changes in context and content of these genes that accompany B cell differentiation, coupled with the biological understanding of the differentiation process, has provided the opportunity to gain a precise understanding of the molecular mechanisms which mediate the process. Experimental data indicate that development of isotype diversity is an intraclonal process that begins with expression of IgM by an immature B cell and leads to a family of plasma cells and memory B cells which express the same immunoglobulin light chains in conjunction with each of the different heavy chain isotypes. Current genetic evidence suggests that isotype switching is accomplished by chromosomal translocation of immunoglobulin gene complexes during differentiation. Further definition of the genetic mechanisms involved in immunoglobulin synthesis and isotype switching may soon provide experimental and clinical immunologists with the capabilities to genetically engineer the system as necessary to either enhance or abolish a specific immunoglobulin isotype. Recent demonstrations that the c-myc proto-oncogene is translocated to the immunoglobulin genetic region in some Burkitt's lymphoma and mouse myeloma cells have provided important clues to the regulation of cellular growth.

GENETIC CONTROL OF THE IMMUNE SYSTEM

Although the functional distinction of lymphocyte populations is convincingly documented, the purpose and mechanisms of their interactions with each other and with macrophages is still being examined. It is clear that B lymphocytes differentiate to become antibody-secreting plasma cells and that differentiated T lymphocyte subsets can exert helper or suppressor effects on B lymphocytes as well as effector functions in transplantation reactions and in cell-mediated immune reactions to various microbial agents. The cooperative and effector functions of T lymphocytes have been found to correlate well with the presence of the cell surface Lyt antigens. It also is known that these functions are controlled and regulated by other cell surface antigens which are products of the genes of the major histocompatibility complex (MHC). The regulatory role of MHC products and the functional roles of other cell surface antigens in the immune response have been defined most extensively in the mouse, although systems analogous to the H–2 component of the murine MHC have been identified in other animals and man.

Evidence obtained in several experimental systems has demonstrated convincingly that efficient physiological interactions among macrophages, T cells, and B cells require that these cells share membrane molecules encoded by the MHC. The genes controlling interactions between T and B lymphocytes are located in the I region of the mouse H–2 complex. Genetic restrictions are also imposed by products of I region on interactions between macrophages and immune T cells.

The molecular basis for genetic restriction of the immune system is the subject of intense interest now that the technology of gene cloning has been applied to the MHC. The complete nucleotide sequence of several MHC genes has been determined and, within the immediate future, it is expected that the complete molecular structure of representatives of the major classes of MHC genes and the protein products encoded by these genes will be available. The structural definition of MHC gene products will provide the opportunity to clarify the molecular basis of genetic restriction of immune responses, as well as of the cell-cell recognition process which can play a key role in transplantation, autoimmunity, and in resistance to a variety of infectious and neoplastic diseases.

REGULATION OF THE IMMUNE SYSTEM

Interest is focused on soluble immunoregulatory products of T lymphocytes. It is clear that soluble helper and suppressor factors are released by their respective cells and that these factors are functional when added to antigen-stimulated cell cultures. The potential use of such soluble immunoregulatory factors in the intact host has served as a stimulus for study of the molecular nature and mechanisms of action of these factors. The possibility of achieving immunoregulation with cellular products is exciting because it avoids the recognized complications of attempted cell transfers. For this reason, among others, efforts to produce soluble immunoregulatory factors in large volumes and in relatively pure forms are increasing. Hybridoma technology is one possible approach which has met with some success in the murine system; a T suppressor hybridoma has been developed and its suppressor factor has been reported to possess functional and chemical properties identical to those of the parent suppressor T cell. Efforts to obtain molecular clones for these products are being vigorously pursued.

Another active area in immunoregulation concerns the unique antigenic determinants or idiotypes associated with the variable regions of antibodies, receptors, and antigen-binding factors. These idiotypic markers constitute a target for the regulatory action of idiotype-specific antibodies, T lymphocytes and other soluble factors. Significant progress has been made recently in elucidating aspects of the regulatory process which involve idiotype/anti-idiotype interactions. Treatment of mice with anti-idiotype antibody has been demonstrated to abolish delayed-type hypersensitive reactivity by the action of suppressor T cells; certain antibody responses also have been found to be inhibited by treating mice with anti-idiotype antibody. The unique specificity of anti-idiotype antibodies adds to their potential for use as immunoregulatory agents. Hybridoma technology is now being employed to prepare monoclonal anti-idiotype antibodies in quantity for subsequent investigation of their immunoregulatory potential.

The related area concerning interleukins also is receiving considerable investigative attention. The interleukins are products of stimulated cells that exert multiple biological effects and regulate immunological and inflammatory host responses by serving as intercellular messengers which modulate cellular functions. Their biological activities are immunologically nonspecific and genetically unrestricted but have proven to be useful to experimental immunologists. Interleukin I has been shown to enhance B lymphocyte antibody production, promote lymphokine production and augment thymocyte proliferation. Interleukin II has been demonstrated to have a variety of biologic activities including, among others, non-specific helper function, T cell growth promoting ability, and thymocyte maturation capability. B cell growth factor has a key role in receptor stimulated proliferation of B cells. As individual molecules are characterized, efforts to prepare monoclonal antibodies against them and against their receptors for use as anti-inflammatory agents are anticipated.

The most extensive study of the interleukins is centered on the T cell growth promoting activity of Interleukin II. The use of Interleukin II as T cell growth promoter has permitted a rapidly developing technology in this area. Cultures of cloned T cells are being developed for large scale production of T cells and products for investigative use. The availability of sufficient quantities of cultured T cell clones now permits the isolation of sub-cellular components for biological and biochemical characterization. The elucidation of molecular mechanisms involved in recognition, restriction, and immunoregulation has been immensely accelerated through the availability of cloned T cell lines.

The past 2 years have been especially notable for the techniques first developed for basic research on mechanisms of host defense that continue to be rapidly applied to methodology that provides useful information about disease pathogenesis and new ideas for disease treatment. Present regulation of the immune system at the clinical-therapeutic level continues to focus on “down-regulation” of apparent “hypersensitivity” with corticosteroids, cytotoxic agents, and the new T lymphocyte suppressing agent, cyclosporine. Additionally, attempts to augment immune responses with a new family of so-called biologic-response modifiers have been initiated. This development has occurred as a result of the availability of quantities of these compounds necessary for controlled clinical trials. Thus, potent immunoregulatory compounds, like interleukins and interferons, so far the most carefully investigated biologic-response modifiers, can be identified and mass produced in cloned tumor cells or in bacteria functioning under recombinant DNA technology.

Three families of interferons, alpha, beta, and gamma, are produced by mammalian cells, including lymphocytes and fibroblasts when these cells are stimulated in the inflammatory process. These compounds have paradoxical effects, in that under different circumstances they can augment and suppress cellular immune function. Two important cell populations seem dependent on these compounds for their normal function. Human monocyte-derived macrophages exert enhanced antimicrobial activity against intracellular pathogens, are primed for tumoricidal activity, and release Interleukin I when exposed to interferons. Large lymphocytes with distinctive cytoplasmic granules (natural killer cells), which seem to be of major importance in preventing dissemination of neoplastic cells, viruses, and other intracellular pathogens, are activated to optimal function by this family of compounds. Clinical trials using these compounds to attempt stimulation of affected cells are under way for a variety of malignancies, including acquired immune deficiency syndrome (AIDS) with opportunistic infection of Kaposi's sarcoma, malignant melanoma, and viral diseases including disseminated herpes simplex. Some malignancies, such as hairy-cell leukemia and several viral infections, seem very responsive to interferons, although large clinical trials are required to substantiate the preliminary data presently available.

Another family of biologic-response modifiers, the interleukins, have a variety of functions, but on the whole seem to up-regulate cell-mediated immunity. Pure Interleukin II has been produced in large quantities and is undergoing clinical trials in patients with the cell-mediated immune deficiency associated with AIDS. This seems like a logical place to begin clinical investigation with this compound, as lymphocytes from patients with AIDS have been shown to have a deficiency of Interleukin II production. Interleukin II also has potential value in the treatment of a variety of other human diseases, particularly viral, fungal, and mycobacterial infections.

Not only do interleukins seem to be potentially useful for treatment in diseases where cell-mediated immunity is deficient, but they may also play a part in disease pathogenesis. In recent studies of sarcoidosis, lung lymphocytes recovered from patients with active interstitial lung disease produced abnormally high levels of Interleukin II. This provides the first good explanation for the proliferation of lung T lymphocytes and the hypergamma-globulinemia that originates at the lung level in sarcoidosis. Another interleukin, termed “alveolar macrophage-derived growth factor,” seems to be distinct from Interleukin I (leukocyte-activating factor) produced by peripheral blood macrophages. The former has been found to be released spontaneously from alveolar macrophages obtained by lung lavage from patients with interstitial pulmonary fibrosis. Also, this compound has fibroblast growth-promoting activity and thus provides a plausible explanation for the increased fibroblast activity and dense pulmonary fibrosis associated with this disease. The stimulus initiating production of this compound is yet to be identified.

Decreased production of Interleukin II is but one of a host of immunologic abnormalities noted in patients with AIDS. As a new disease, AIDS has occupied center stage position in immunologic investigation over the past several years. Not only is there an urgency to find the cause and cure, but there is also an unprecedented opportunity to study microbial and tumor host defense mechanisms at a time when a variety of new tools, including monoclonal antibodies that identify subsets of human leukocytes, are available. AIDS patients, usually from homosexual, hemophiliac, or intravenous drug user groups, have a disease that appears to be transmitted by semen and blood. The clinical manifestations can include syndromes varying from asymptomatic lymphadenopathy to wasting disease with opportunistic infection and or malignancy. The complicating infections are in many cases similar to those experienced by patients with congenital deficiencies of cell-mediated immunity. For instance, the DiGeorge syndrome and AIDS both are associated with decreased numbers and function of T helper inducer lymphocytes. Now that most of the immunologic abnormalities present have been characterized, major efforts are directed at understanding the mechanisms that may cause the disappearance of these cells from the peripheral blood and the isolation and characterization of the sexually transmitted agent(s) that cause the disease.

Newer findings at the cellular level are also providing valuable clinical information in immediate hypersensitivity diseases. For instance, the mast cell-basophil cell series so central to diseases of immediate hypersensitivity has been found to produce not only such familiar bioactive amines as histamine, but also newly discovered cell-membrane-derived arachidonic acid metabolites with powerful inflammatory actions. The cyclooxygenase pathway of arachidonic acid metabolism produces the prostaglandin family of compounds and the lipoxygenase pathway produces the leukotriene family. Prostaglandins of the F-series are potent bronchoconstrictors as are certain leukotrienes, which are especially effective in increasing vascular permeability and attracting inflammatory cells into tissues. Perturbations of the normal levels of these compounds in tissues, such as may occur in some asthmatics after aspirin ingestion or prostaglandin infusion for obstetrical purposes, may result in acute bronchospasm. The production of these arachidonic acid metabolites is generally decreased by corticosteroids and may explain the salutory effects of corticosteroid treatment in chronic allergic diseases like bronchial asthma. An understanding of the effects of these compounds on human tissues is also changing older concepts of allergy, such that we now realize that the release of mast cell-derived mediators may result in clinical events that last not just for minutes but for many hours. Thus, certain leukotriene compounds liberated from lung mast cells seem to be logical candidates as mediators of severe nocturnal bronchospasm, which frequently occurs six to eight hours after allergen inhalation, and the late, so-called, "second wave" or recurrent anaphylactic reactions, which occur after a single allergen exposure.

Mast cell-derived chemotactic factors are also being recognized as important in human diseases. Earlier studies, which showed an increase in skin or respiratory tract eosinophils after cutaneous or respiratory allergic reactions, respectively, have been supplemented with a more complete analysis of the cellular effects of mast cell- and basophil derived IgE-mediated events. Chemotactic factors for neutrophils and eosinophils, their biologic effects, and their importance in disease pathogenesis are now better understood. It seems likely that mast cells and their various chemotactic factors, acting at the host-environment interfaces in the skin, respiratory tract, and gastrointestinal tract, provide a sensitive defense system for first contact of the host with parasitic organisms and foreign particulate matter. When mast cells are activated, there occurs not only the classic vasopermeability and smooth muscle vasoconstrictor effects mediated by arachidonic acid metabolites and bioamines, but also the generation of chemotactic factors. These factors attract and facilitate the ingress of phagocytes into sites of antigen deposition so as to dispose of the foreign material. If, on the other hand, mast cell stimulation is excessive in degree or persistent, excessive mast cell mediators and chemotactic factors may be responsible for continuing inflammation. This mechanism most likely explains the inflammatory cell infiltrate in the bronchial wall of patients with status asthmaticus, the origin of which has been a long-standing mystery.

APPLICATIONS OF TECHNOLOGY

Realization of the practical results of these fundamental studies is, for the most part, still to come. However, the applications that have developed amply justify the expectation that these advances will have a profound effect on the understanding and treatment of disease states. Hybridoma technology, for example, already has supplied a variety of clinically useful reagents. Its value and potential has been recognized by the Award of the Nobel Prize in Medicine for 1984 to Drs. Cesar Milstein and Georges J. F. Kohler.

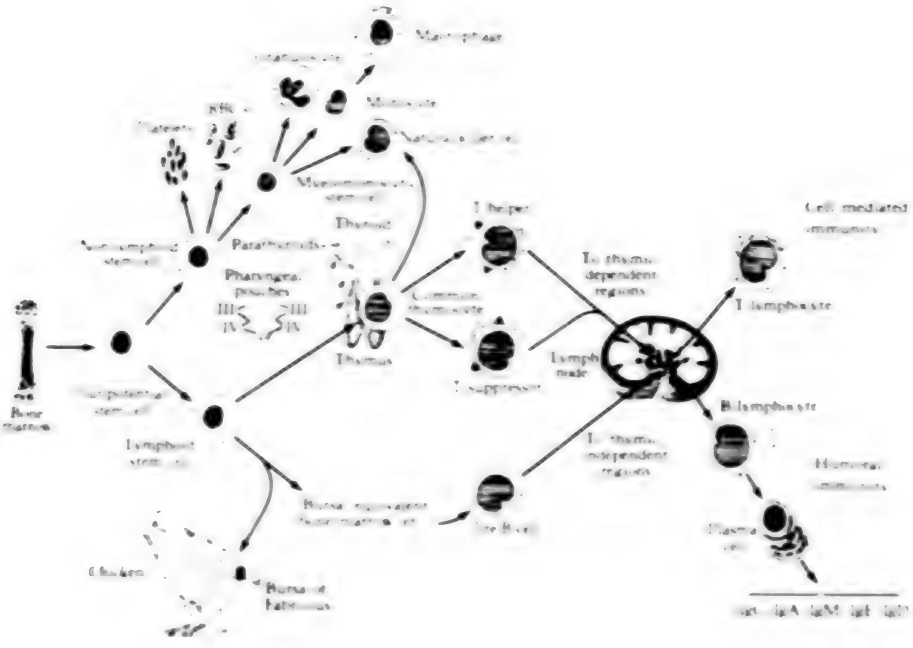
The commercially available OKT series of monoclonal antibodies can effectively distinguish human peripheral blood lymphocytes on a functional or differentiated basis. Human helper and suppressor T lymphocytes, distinguished by these antibodies have been employed in the clinical diagnoses of various immunologic disorders. Acute lymphoblastic leukemias have been characterized on the basis of differential expression of OKT defined antigens, and abnormal T cell levels have been detected in patients with multiple sclerosis and systemic lupus erythematosus. Promising results are appearing with the use of monoclonal antibodies as diagnostic and therapeutic agents in viral infections. Monoclonal antibodies against influenza and rabies viruses have been found to detect serologic differences between isolates indistinguishable by previously available diagnostic antisera. Monoclonal anti rabies antibodies have been demonstrated to be dramatically effective in protecting mice against lethal infections.

Monoclonal antibodies also have the potential to improve the diagnosis and treatment of cancer. Antibodies against tumor-associated antigens are being developed for imaging small tumors and have been administered clinically in the treatment of tumors. Such antibodies have also been conjugated to toxins or cytotoxic drugs and have been used to treat targeted, experimental tumors. It is reasonable to expect that, in the near future, applications of hybridoma technology for clinical diagnosis will be extensive and will offer extremely useful approaches to therapy of a variety of immunologic, infectious, and neoplastic diseases.

At the more basic level, monoclonal antibodies have been employed for study of biologically important molecules that previously had escaped detection, such as differentiation antigens on lymphocytes that help to distinguish different stages of cell development and to define subsets that perform discrete functions. It now is possible to phenotype the surface antigens of all cell lineages. These reagents provide new avenues for dissecting molecular aspects of immunoglobulins, such as the nature of the antigen-combining site and the three dimensional structure of the antibody molecule. New gene products also have been detected. As an example, the human class II major histocompatibility complex antigens, associated with increased susceptibility to such diseases as rheumatoid arthritis, juvenile diabetes, and multiple sclerosis, were poorly defined before monoclonal antibodies were available. The different loci and alleles for these antigens are being enumerated in the human and, as a result, new disease associations are being described.

INTERACTIONS AND EXCHANGES

Discussions between U.S. and Japanese investigators have been vigorous and extensive at recent Board-sponsored



Schematic representation of ontogeny of immune response, showing differentiation of progenitor cells into hematopoietic and immunocompetent cells.

From *Immunology III* by J. A. Bellanti. W. B. Saunders Co., Philadelphia, 1985.

workshops and clearly support the use of this meeting format as a very effective means for exchange of scientific opinion and information and for establishing fruitful collaborations.

At their recent joint meetings, the U. S. and Japanese Immunology Board members have reviewed current and planned future activities designed to facilitate the exchange of research materials and information between the two countries. The Boards endorsed the continued availability of hybridoma cell lines from banks maintained at the American Type Culture Collection and supported by the National Institute of Allergy and Infectious Diseases and the National Cancer Institute. In addition, the establishment of a Genetic Sequence Data Bank, supported by the National Institute of Allergy and Infectious Diseases as well as other components of the National Institutes of Health and other U. S. Government agencies, was noted. This resource serves a repository for all published nucleic acid sequences greater than 50 nucleotides in length; the sequence data are available to the international scientific community on computer readable magnetic tape or by limited on-line access. Also available for investigators are research reagents maintained in banks supported by the National Institute of Allergy and Infectious Diseases, which include extensive collections of antisera directed against murine H-2 antigens and cell surface alloantigens, human histocompatibility antigens, and a variety of allergens and allergenic extracts.

The Boards also encouraged the development of a computer-based Hybridoma Data Bank which will store and distribute information on the existence and availability of hybridomas and their products. A network of computer centers, sharing data stored on magnetic tape, is being established to respond by mail to inquiries from investigators. The Bank's central operations are located at the American Type Culture Collection; agreement has been reached to establish a computer center in Japan to furnish service to the entire Far East and negotiations are in process to place a similar center in Europe. This effort is being supported jointly by the International Union of Immunologic Societies, the Committee on Data for Science and Technology of the International Council of Scientific Unions, the World Health Organization, and relevant scientific agencies of several national governments, including Japan and the United States.

FUTURE GOALS

The highly interactive and successful activities of the Immunology Board during its relatively short history indicate that its broad goals and guidelines were well founded and require little or no modification. The Board, subsequently, intends to vigorously support and encourage its objectives for the future. The roundtable and workshop formats for joint conferences, effective in both promoting exchanges of information and developing collaborative efforts, will be continued. Similarly, the acquisition of research resources and reagents to facilitate research efforts will receive continued encouragement and endorsement. At earlier meetings, the Board recognized the merits of an exchange system between investigators in U. S. and Japanese laboratories and will continue to endorse the development of a mechanism which would implement and support such a plan.

BIBLIOGRAPHY

Allison, J.P., L. Ridge, J. Lund, J. Gross-Pelose, L. Lanier, and B.W. McIntyre. The murine T cell antigen receptor and associated structures. *Immunol. Rev. 81*:145-160, 1984.

Altman, A. and D.H. Katz. The biology of monoclonal lymphokines secreted by T cell lines and hybridomas. *Adv. Immunol. 33*:73-166, 1982.

Beer, D.J., S.M. Matloff, and R.E. Rocklin. The influence of histamine on immune and inflammatory responses. *Adv. Immunol. 35*:209-268, 1984.

Cantrell, D.A. and K.A. Smith. The interleukin-2 T cell system: A new cell growth model. *Science 224*:1312-1316, 1984.

Cupps, T.R. and A.S. Fauci. Corticosteroid-mediated immunoregulation in man. *Immunol. Rev. 65*:133-155, 1982.

Davis, M.M., Y. Chien, N.R.J. Gascoigne, and S.M. Hedrick. A murine T cell receptor gene complex: Isolation, structure and rearrangement. *Immunol. Rev. 81*:235-258, 1984.

De Franco, A.L., J.T. Kung, and W.E. Paul. Regulation of growth and differentiation in B cell subpopulations. *Immunol. Rev. 64*:161-182, 1982.

Dinarello, C.A. Interleukin-1 and the pathogenesis of the acute-phase response. *New Eng. J. Med. 311*:1413-1418, 1984.

Fathman, C.G. and M. Kimoto. Studies utilizing murine T cell clones. Ir genes, Ia antigens and MLR stimulating determinants. *Immunol. Rev. 54*:57-80, 1981.

Gattings, W.E., H. Kubagawa, and M.D. Cooper. A distinctive pattern of B cell immaturity in perinatal humans. *Immunol. Rev. 57*:108-126, 1981.

Gillis, S. and J. Watson. Interleukin-2 dependent culture of cytolytic T cell lines. *Immunol. Rev. 54*:81-110, 1981.

Hannum, C., J.H. Freed, G. Tarr, J. Kappler, and P. Marrack. Biochemistry and distribution of the T cell receptor. *Immunol. Rev. 81*:161-176, 1984.

Haynes, B.F. Human T lymphocyte antigens as defined by monoclonal antibodies. *Immunol. Rev. 57*:127-161, 1981.

Herzenberg, L.A., K. Hayakawa, R.R. Hardy, T. Tokuhisa, V.T. Oi. Molecular, cellular and systematic mechanisms for regulating Ig CH expression. *Immunol. Rev. 67*:5-32, 1982.

Howard, M., K. Nakanishi, and W.E. Paul. B cell growth and differentiation factors. *Immunol. Rev. 78*:185-210, 1984.

Huber, B.T. B cell differentiation antigens as probes for functional B cell subsets. *Immunol. Rev. 64*:57-80, 1982.

Kemp, J.D., J.W. Rohrer, and B.T. Huber. Lyb 3: a B cell surface antigen associated with triggering secretory differentiation. *Immunol. Rev. 69*:127-140, 1983.

Kincade, P.W. Formation of B lymphocytes in fetal and adult life. *Adv. Immunol. 31*:177-246, 1981.

Kornbluth, J., D.M. Silver, and B. Dupont. Cloning and characterization of primary alloreactive human T-lymphocytes. *Immunol. Rev. 54*:111-156, 1981.

Lanier, L.L., E.G. Engleman, P. Gatenby, G.F. Babcock, N.L. Warner, and L.A. Herzenberg. Correlation of functional properties of human lymphoid cell subsets and surface marker phenotypes using multiparameter analysis and flow cytometry. *Immunol. Rev. 74*:143-160, 1983.

Perlmutter, R.M., S.T. Crews, R. Douglas, G. Sorensen, N. Johnson, N. Nivera, R.J. Gearhart, and L. Hood. The generation of diversity in phosphorylcholine-binding antibodies. *Adv. Immunol. 35*:1-38, 1984.

Reinherz, E.L., S.C. Meuer, and S.F. Schlossman. The human T cell receptor: Analysis with cytotoxic T cell clones. *Immunol. Rev. 74*:83-112, 1983.

Rock, K.L. and B. Benacerraf. MHC-restricted T cell activation: Analysis with T cell hybridomas. *Immunol. Rev. 76*:29-58, 1983.

Rogers, J. and R. Wall. Immunoglobulin RNA rearrangements in B lymphocyte differentiation. *Adv. Immunol. 35*:39-60, 1984.

Rosen, F.S., M.D. Cooper, and R.J. Wedgwood. The primary immunodeficiencies: first of two parts. *New Eng. J. Med. 311*:235-242, 1984.

Rosen, F.S., M.D. Cooper, and R.J. Wedgwood. The primary immunodeficiencies: second of two parts. *New Eng. J. Med. 311*:300-310, 1984.

Shoenfeld, Y. and R.S. Schwartz. Immunologic and genetic factors in autoimmune diseases. *New Eng. J. Med. 311*:1019-1029, 1984.

Smith, K.A. and F.W. Ruscetti. T-cell growth factor and the culture of cloned functional T cells. *Adv. Immunol. 31*:137-176, 1981.

Stastny, P., E.J. Ball, P.J. Dry, and G. Nunez. The human immune response region (HLA-D) and disease susceptibility. *Immunol. Rev. 70*:113-154, 1983.

Storb, R. and E.D. Thomas. Allogeneic bone-marrow transplantation. *Immunol. Rev. 71*:77-102, 1983.

Subbarao, B. and D. E. Mosier. Lyb antigens and their role in B lymphocyte activation. *Immunol. Rev.* *69*:81-98, 1983.

Swain, S. L. T cell subsets and the recognition of MHC class. *Immunol. Rev.* *74*:129-142, 1983.

Thomas, Y., L. Rogozinski, and L. Chess. Relationship between human T cell functional heterogeneity and human T cell surface molecules. *Immunol. Rev.* *74*:113-128, 1983.

Uhr, J. W. Immunotoxins: Harnessing nature's poisons. *J. Immunol.* *133*:i-x, 1984.

Unanue, E. R. The regulatory role of macrophages in antigenic stimulation. Part two: Symbiotic relationship between lymphocytes and macrophages. *Adv. Immunol.* *31*:1-136, 1981.

Vitetta, E. S., K. A. Krolick, and J. W. Uhr. Neoplastic B cells as targets for antibody-ricin A chain immunotoxins. *Immunol. Rev.* *62*:159-184, 1982.

Waldmann, T. A. and S. Broder. Polyclonal B-cell activators in the study of the regulation of immunoglobulin synthesis in the human system. *Adv. Immunol.* *32*:1-65, 1982.

Joint Scientific Conferences

IMMUNOLOGY BOARD

Date: May 11-13, 1982

Place: Bethesda, Maryland

Summary: The first official joint conference of the newly formed Immunology Boards was held on the NIH campus and was concerned with "Applications of Recent Advances in Fundamental Immunology to the Study of the Human Immune Response." The conference was organized into four scientific sessions, each containing four survey presentations, and a fifth session, devoted to a round table discussion of new approaches in human immunology. Topics discussed in the scientific sessions included molecular and functional studies of important genes of the human immune system, monoclonal antibodies in the diagnosis and treatment of human disease, growth factor action in human cells, and long-term cell lines and somatic cell hybrids.

Date: August 21, 1983

Place: Kyoto, Japan

Summary: Immediately before the Fifth International Congress of Immunology, the Boards sponsored a one-day workshop, concerned with B cell activation and differentiation factors, which was attended by approximately 40 investigators, principally from the United States and Japan. The workshop led to the development of a uniform nomenclature for the lymphokines that regulate the activation, growth, and differentiation of B lymphocytes, approaches for exchange of materials, and the establishment of collaborative studies of materials between participating laboratories in the United States and Japan, so that more rapid progress can be made in defining these biologically important lymphocyte products.

Date: August 28-31, 1983

Place: Susono City, Japan

Summary: Immediately following the Fifth International Congress, the Boards sponsored a 3-day meeting which was organized as a workshop on newer technologies for application in immunologic research. Approximately 45 Japanese immunologists, working at either the post-doctoral or junior faculty level, were selected by the Japanese Immunology Board to attend the workshop; 10 U.S. and 8 Japanese senior immunologists served as "faculty." Topics for extensive discussion included lymphocyte growth and differentiation factors, immunoregulatory factors, immunogenetics and molecular genetic technology, and ontogeny and differentiation of B and T cells.

Date: January 23-25, 1985

Place: Stanford, California

Summary: Immediately following the Midwinter Conference of Immunologists, the Boards sponsored a two and one-half day workshop on "Lymphocyte Receptors and Growth Regulation" on the University campus. The meeting was organized into five round-table sessions, devoted to the topics of lymphocyte differentiation, lymphocyte recognition mechanisms, chemistry of lymphocyte receptors, molecular biology of antigen binding and growth factor receptors, and growth regulation of lymphocytes. Approximately 40 investigators attended the meeting; 17 U.S. and 9 Japanese participants served as speakers.

U.S.-Japan Cooperative Medical Science Program

History of Program Activities

Program Activity	Initiated	Completed
Meeting of Prime Minister Sato and President Johnson	January 12-13, 1965 Washington, D.C.	–
Joint Planning Committee Meeting	April 19-21, 1965 (Tokyo)	–
First Joint Committee Meeting and Panels' Conference	October 4-7, 1965 Honolulu	–
Subcommittee on Program Review and Planning	1970 (Tokyo)	
Environmental Symposium	(Tokyo)	1972
Environmental Panel	1972	
Second Five Year Report	1974	1975
Binational Symposium	(Tokyo)	1976
Hepatitis Symposium	(Tokyo)	1978
Hapatitis Panel	1979	
Third Five Year Report 1975-1980	1979	1980
Immunology Symposium	(Tokyo)	1980
Immunology Board	1981	
Parasitic Diseases Symposium	(Bethesda)	1983
Fourth Five Year Report 1980-1985	1984	1985
Malnutrition Symposium	(Bethesda)	1985

History of Panel Reviews

1971–1985

Panel Reviews*	**Initiated**	**Completed**
Leprosy and Parasitic	1971	1972
Cholera and Tuberculosis	1972	1973
Malnutrition and Viral	1973	1974
Leprosy and Tuberculosis	1976	1977
Viral and Parasitic	1977	1978
Environmental	1978	1979
Cholera and Malnutrition	1979	1980
Leprosy and Parasitic	1981	1982
Viral Diseases and Tuberculosis	1982	1983
Hepatitis and Environmental	1984	1985
Malnutrition	1985	

---

*Formal reviews initiated with the formation of the Subcommittee on Program Review and Planning in 1970

END

09-18-85